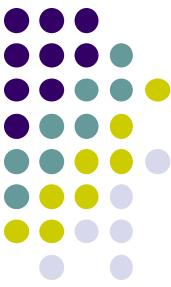


Ovarian Cancer in the Genomics Era

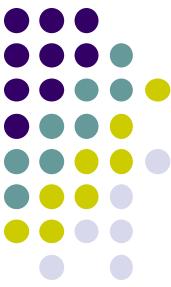
Christina M. Annunziata, MD, PhD

Women's Malignancies Branch
National Cancer Institute
Bethesda, MD



Cancer Genomics

- Study of the genome
 - Chromosomes
 - Gene expression
 - Global analysis (not individual entities)



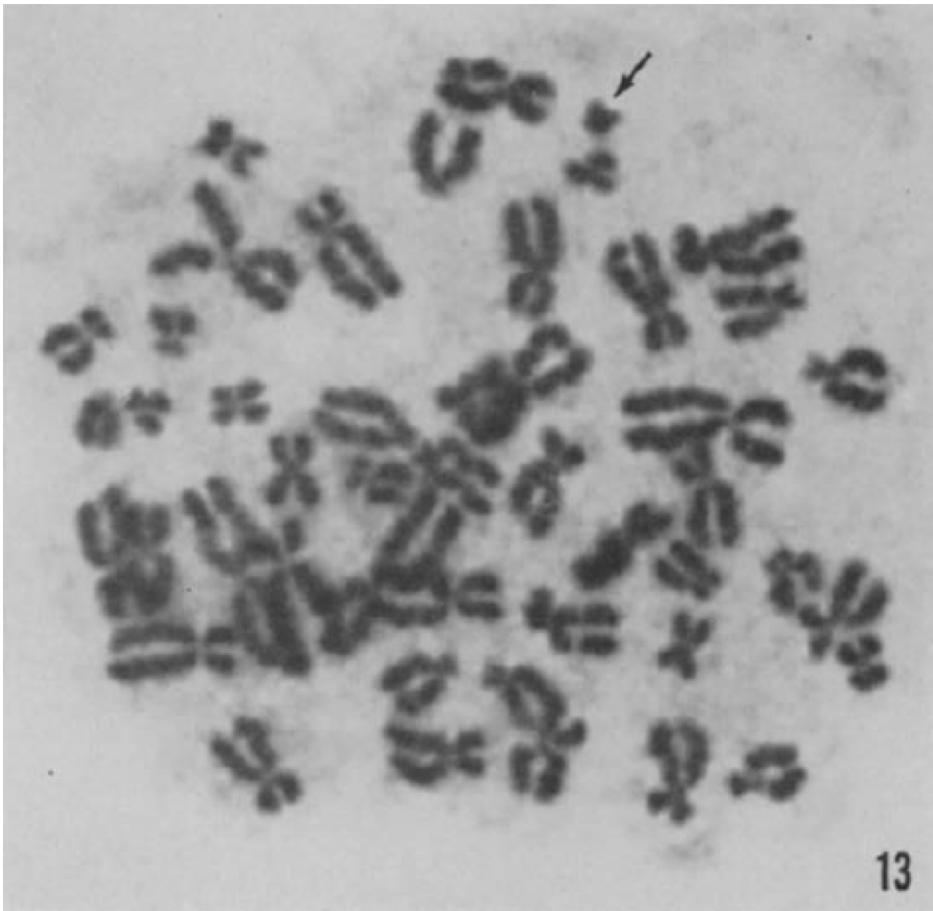
The Genomics Era

- 1959 – Nowell and Hungerford
 - Study of chromosomes
 - Identified recurrent abnormality
 - Philadelphia chromosome
 - Chronic leukemia



The Genomics Era

- 1959 – Nowell and Hungerford





Genomics Era

The Genomics Era

- 1973 – Janet Rowley

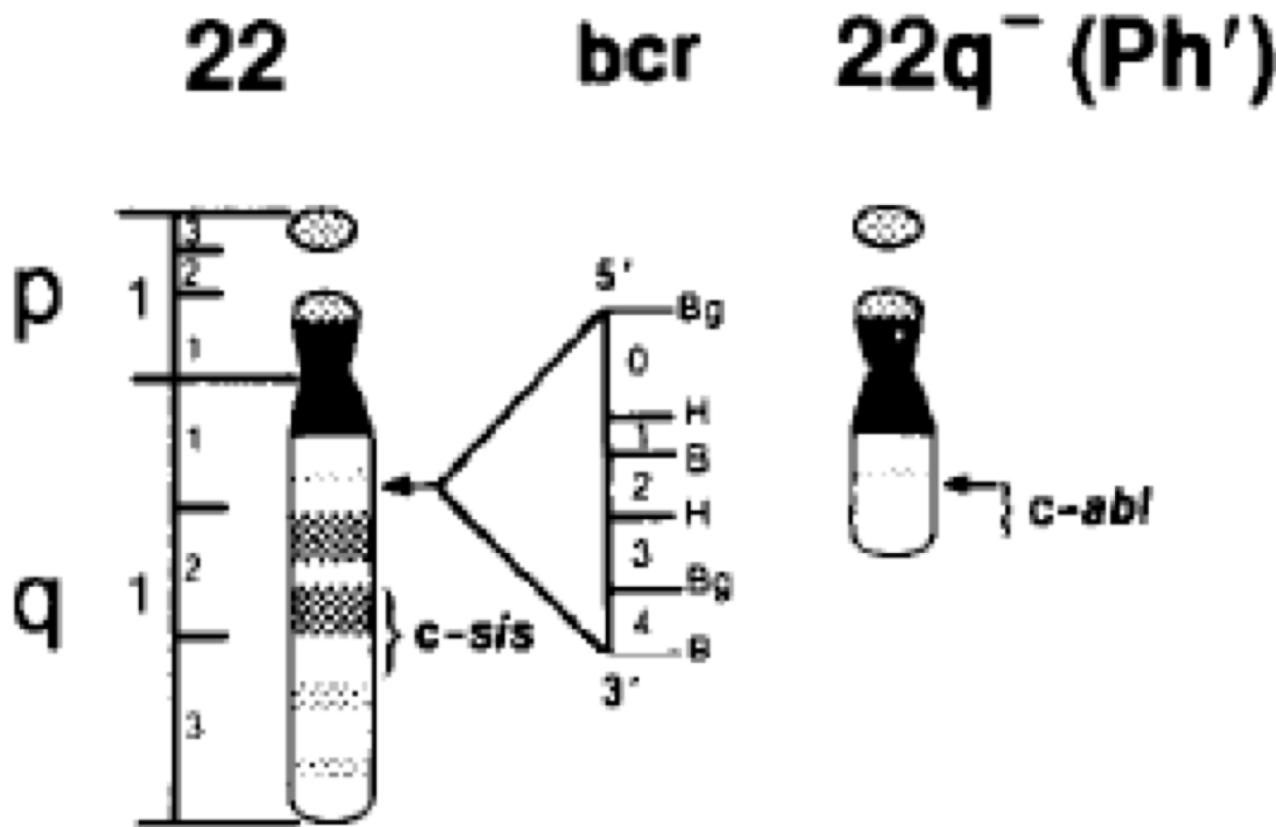
Table 1 Summary of Chromosomal Analysis

Case	Age (yr)	Duration of CML (yr)	Karyotype ^{*6,7}
1§	72	6	46,XY,9q+,22q-
2§	29	3½	48,XY,9q+,+C,+mar,-17,+?F,22q-
3§	37	3½	46,XY,9q+,22q- 50,XY,9q+,+8,+C,+mar,22q-, +22q- 50,XY,9q+,+8,+C,+mar,22q-, +22q-
4§	71	1½	46,XX,9q+,+mar,-17,22q- 47,XX,9q+,+C,+mar,-17,22q-
5§‡	51	2½	48,XY,9q+,+mar,22q-,+22q-
6	45	2 mo	46,XX,9q-1,22q-
7	25	1	46,XX,9q+,22q-
8	18	3	46,XX,9q+,22q-
9	64	3½	46,XX,9q+,22q-



The Genomics Era

- 1984 – Groffen – BCR-ABL

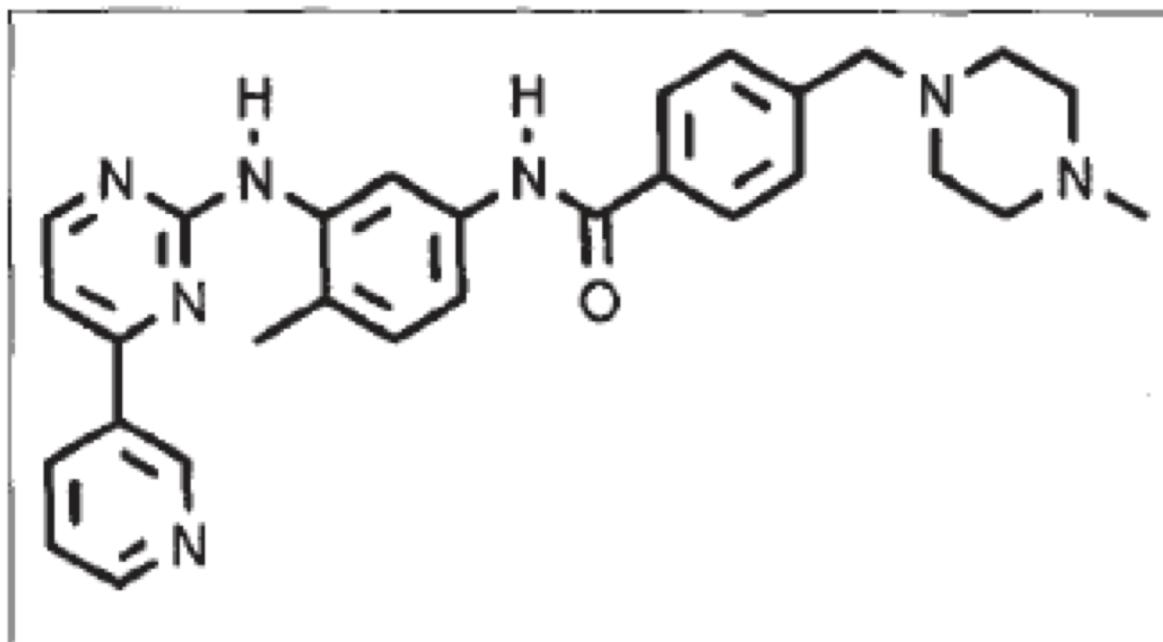




The Genomics Era

- 1996 – Drucker – blocking ABL

Fig. 1 Structure of CGP 57148.





Functional Genomics

- What part of the genome is functional
- Causes an effect
- Transforms normal cells into cancer
- Looking for “driver” alterations



Functional Genomics

- 1981 – Shih – discovery of Her2/neu

**Transforming genes of carcinomas
and neuroblastomas
introduced into mouse fibroblasts**

**Chiaho Shih, L. C. Padhy, Mark Murray
& Robert A. Weinberg**



Functional Genomics

- 1984 – Schechter – neu and EGFR

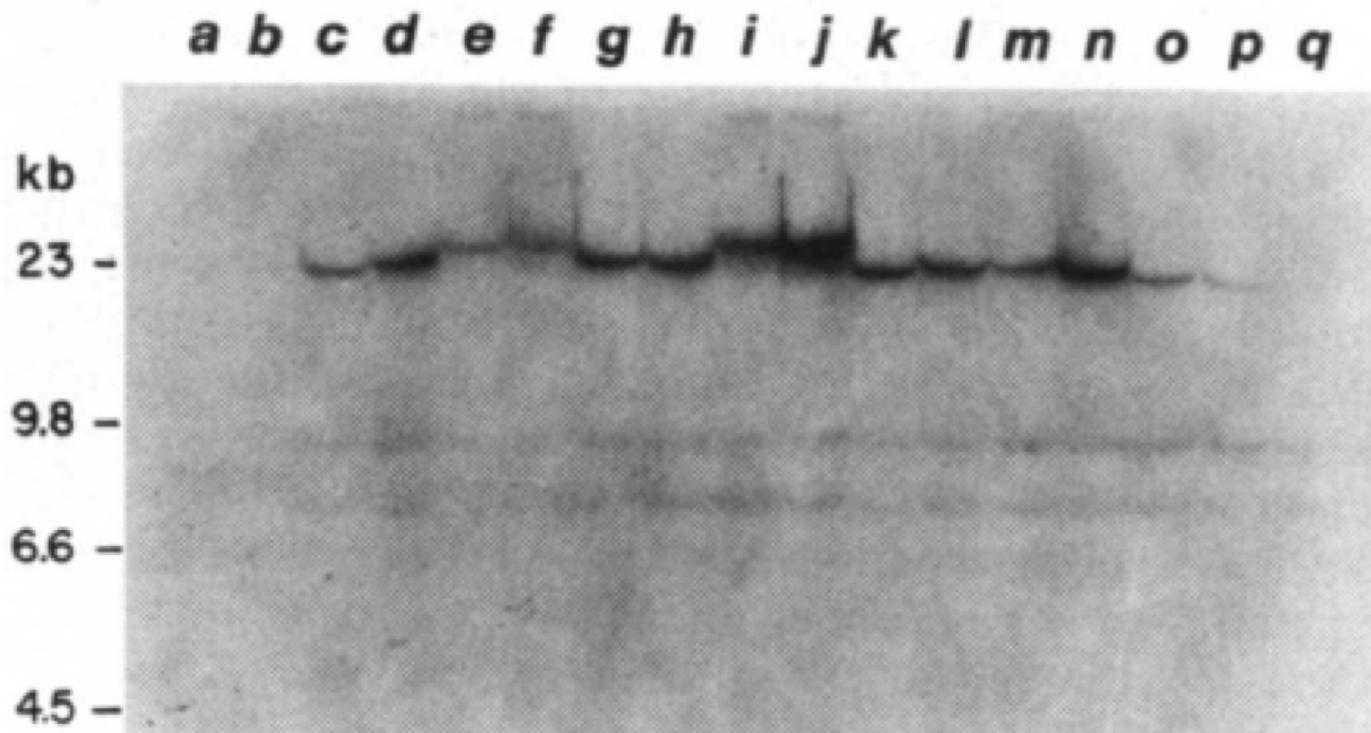
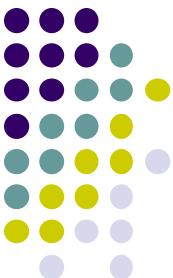
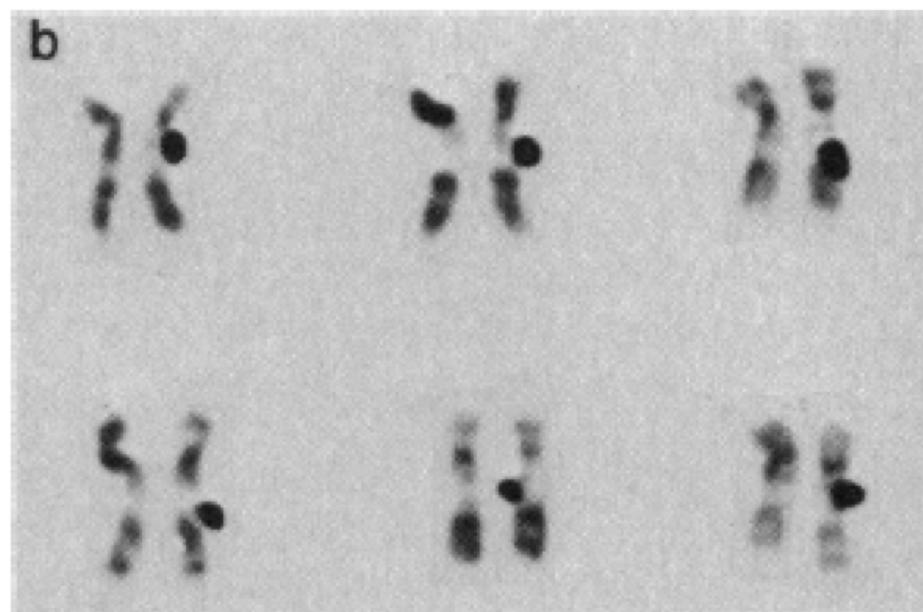
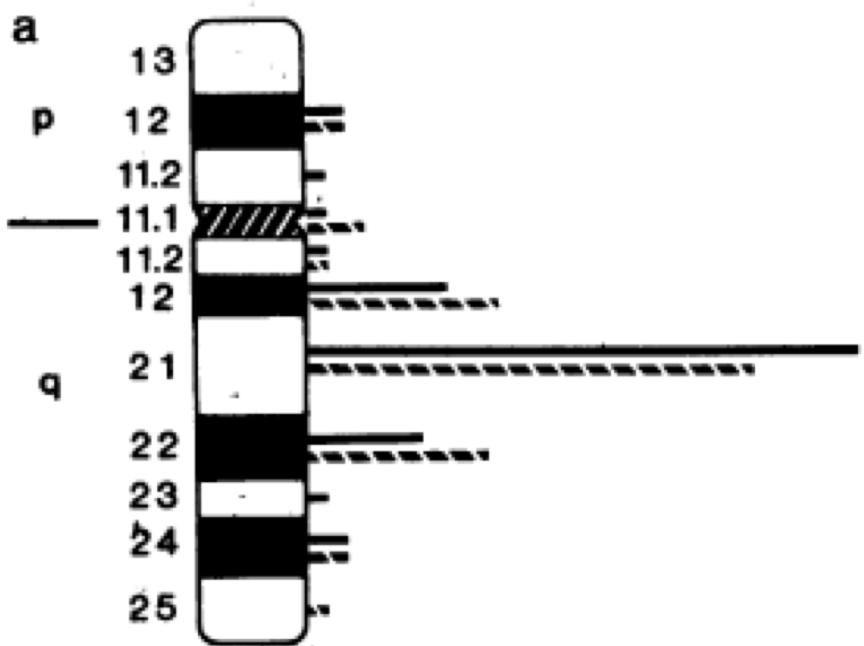


Fig. 1 Southern blot analysis of *erb-B*-related sequences in NIH 3T3 cells transformed with rat neuro/glioblastoma DNAs;



Functional Genomics

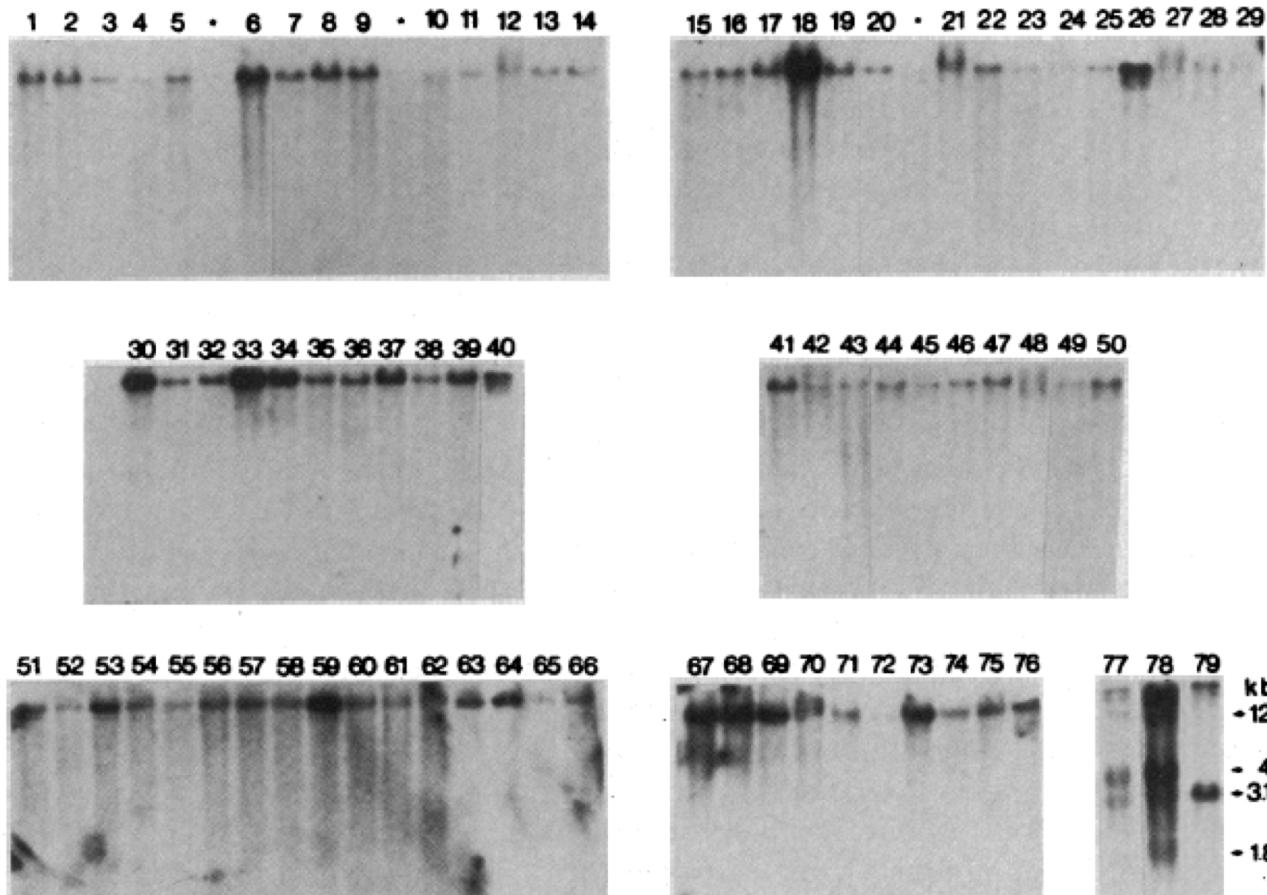
- 1985 – Coussens – Her2 on chromosome 17





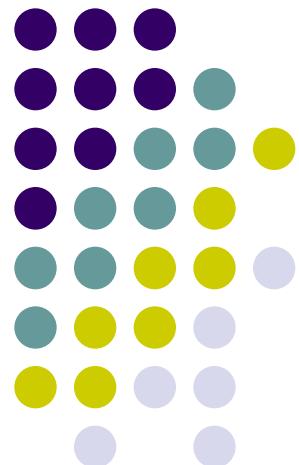
Functional Genomics

- 1987 – Slamon – HER2 in breast cancer



Using genomics to study ovarian cancer

Do we have any “drivers”?

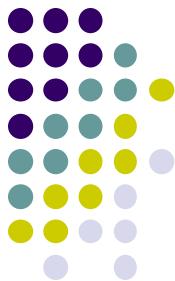




Ovarian Cancer

- Most lethal gynecologic malignancy in the US
 - >16,000 deaths/yr
 - 5th most common cancer death for women
- 70% diagnosed with advanced disease
- < 35% of advanced stage patients alive at 5y

Ovarian Cancer Stages



Ovarian Cancer



Stage	Description	Incidence	Survival
I	Confined to ovaries	20%	90%
II	Confined to pelvis	5%	65%
III	Spread IP or nodes	58%	45%
IV	Distant metastases	17%	<5%

Treatment for Newly Diagnosed Ovarian Cancer



- Complete surgical staging
- Optimal reductive surgery
- Chemotherapy
- *Clinical Trials*

The State of Treatment for Newly Diagnosed Ovarian Cancer



- Complete surgical staging
- Optimal reductive surgery
- **Chemotherapy**
 - Platinum = cisplatin or carboplatin
AND
 - Taxane = paclitaxel or docetaxel
 - *Intraperitoneal if Stage III, optimal reduction*
- *Clinical Trials*



Treatment and Outcome

Treatment and Outcome for Advanced Ovarian Cancer

ALKYLATORS

CISPLATIN/ALKYLATOR COMBINATIONS

INTRAPERITONEAL

1960

1980

2000

1970

CISPLATIN

1990

PACLITAXEL/
CARBOPLATIN

0

5%

15%

35%

40%

1960

1970

1980

1990

2000

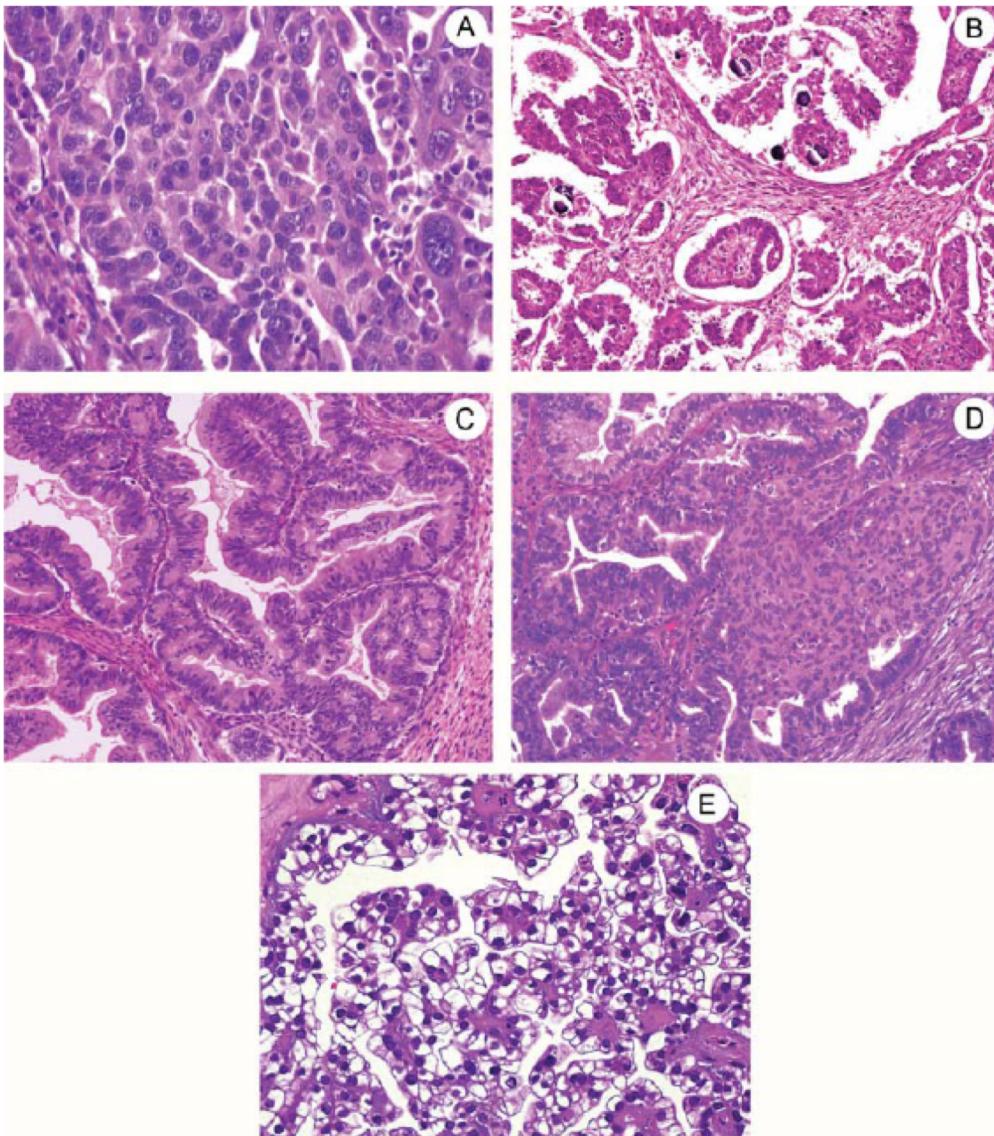
5 YR SURVIVAL ADVANCED DISEASE



Ovarian Cancer

Prevalence

- Serous – 80%
- Endometrioid – 10%
- Clear cell – 5%
- Mucinous – 3%
- Other – 2%





Ovarian Cancer



Ovarian Cancer

Prevalence

- Serous – 80%
- Endometrioid – 10%
- Clear cell – 5%
- Mucinous – 3%
- Other – 2%

Tissue of origin

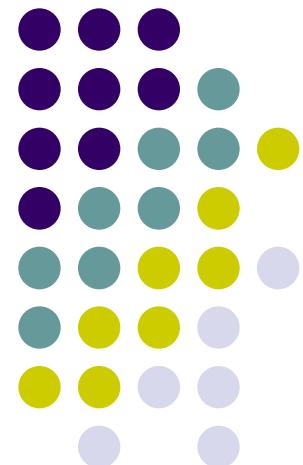
- Fallopian tube?
 - Serous
- Endometriosis?
 - Endometrioid and clear cell
- Mullerian epithelium
 - Extra-uterine



Ovarian Cancer

- Increasing our understanding about the biological and biochemical events underlying ovarian cancer progression will create avenues for new treatments
- Can we use Genomics?

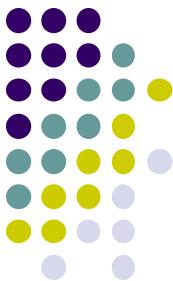
Clear cell, Endometrioid





Clear Cell cancers

- 5-10% of all cases (serous = 70%)
- Worse response to standard chemotherapy
- Associated with endometriosis (up to 40%)



Clear cell ovarian cancer



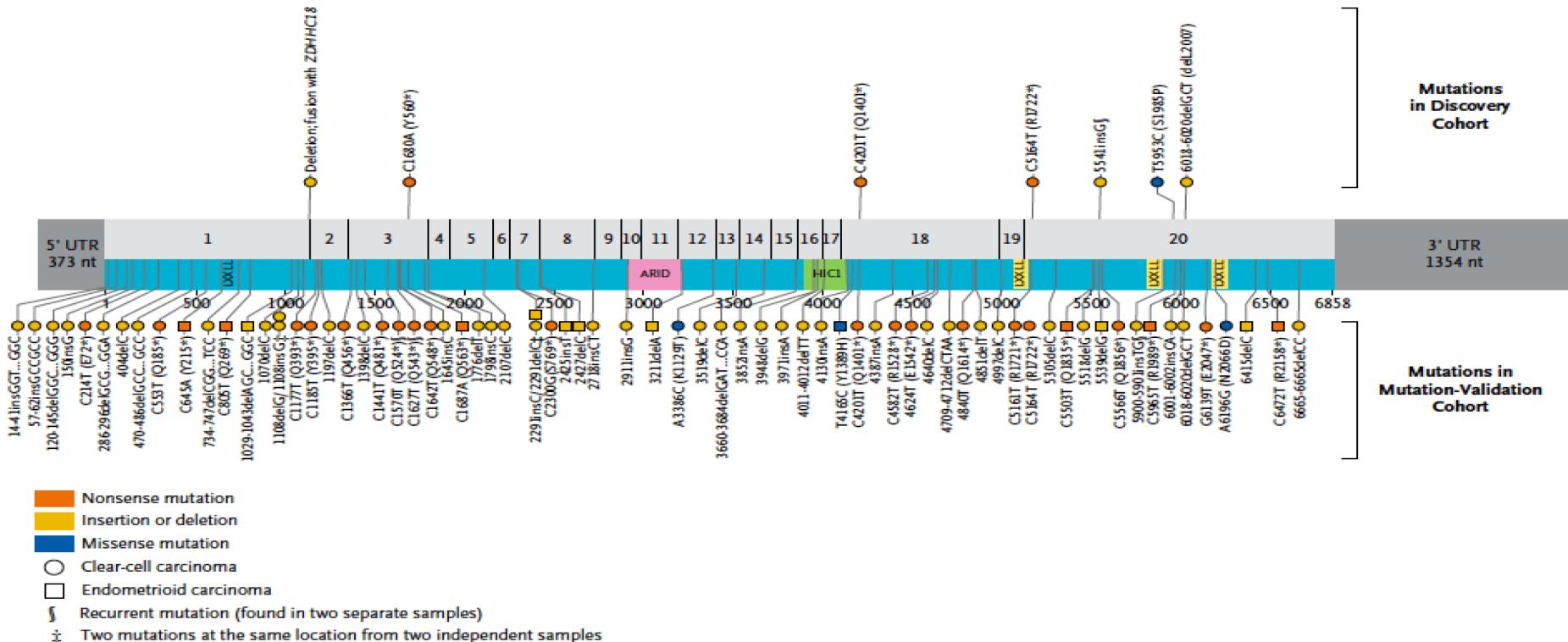
Clear cell OC – genomics

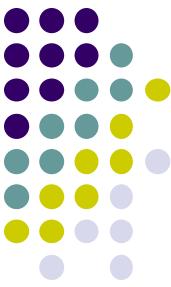
- Sequenced RNA from 18 clear cell ovarian cancers, and one cell line (discovery)
- Sequenced DNA exons from 210 samples
 - 101 more clear cell, 33 endometrioid, 76 serous, 1 more clear cell line (validation)
- Immunostain 455 more samples
 - 132 clear cell, 125 endometrioid, 198 serous



ARID1A mutations in clear cell

ARID1A mutations in clear cell





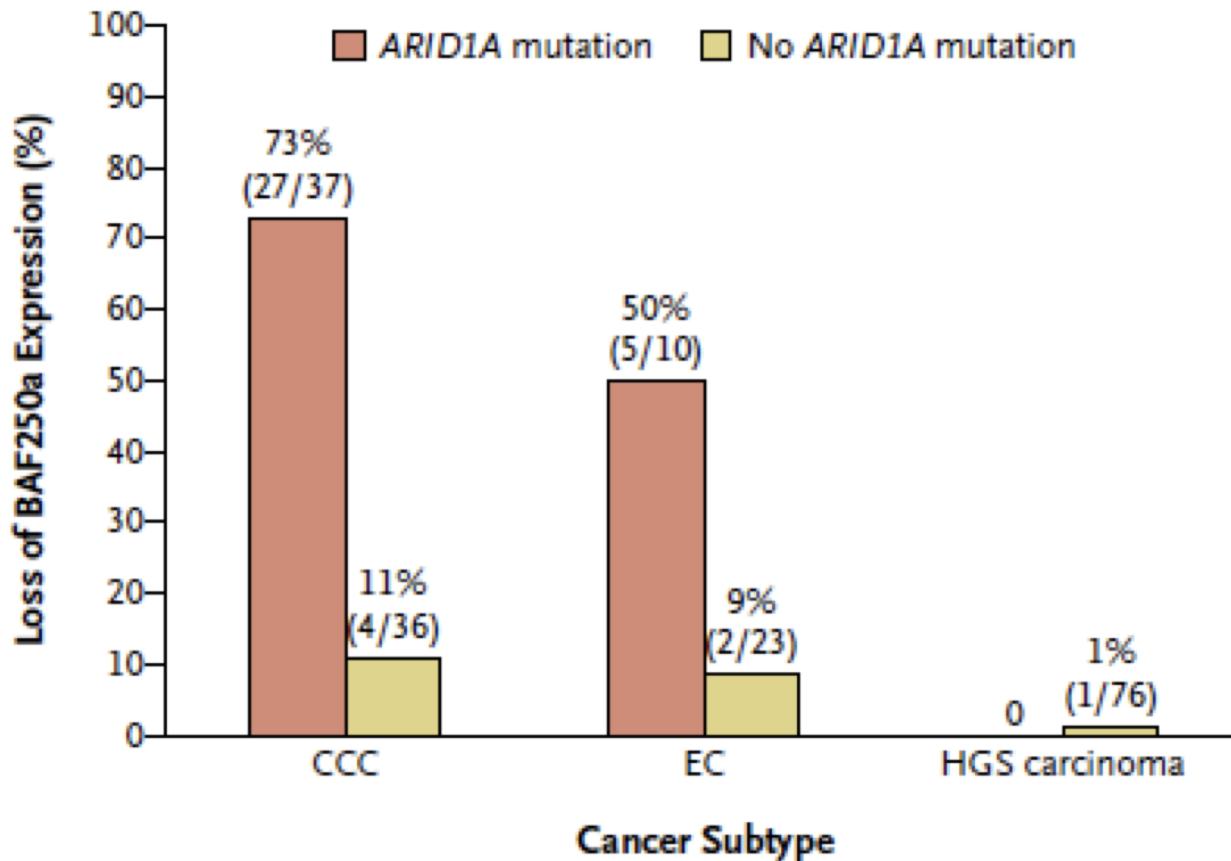
ARID1A

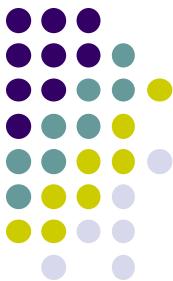
- SWI-SNF chromatin remodeling complex
- Mutated in breast cancer, lung cancer
- 1p36: deleted 6% of all cancers
- Tumor suppressor gene?



ARID1A mutations

A

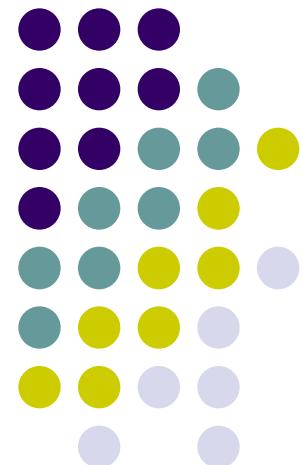


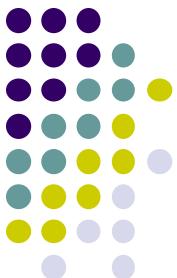


Clear cell and endometrioid cancer

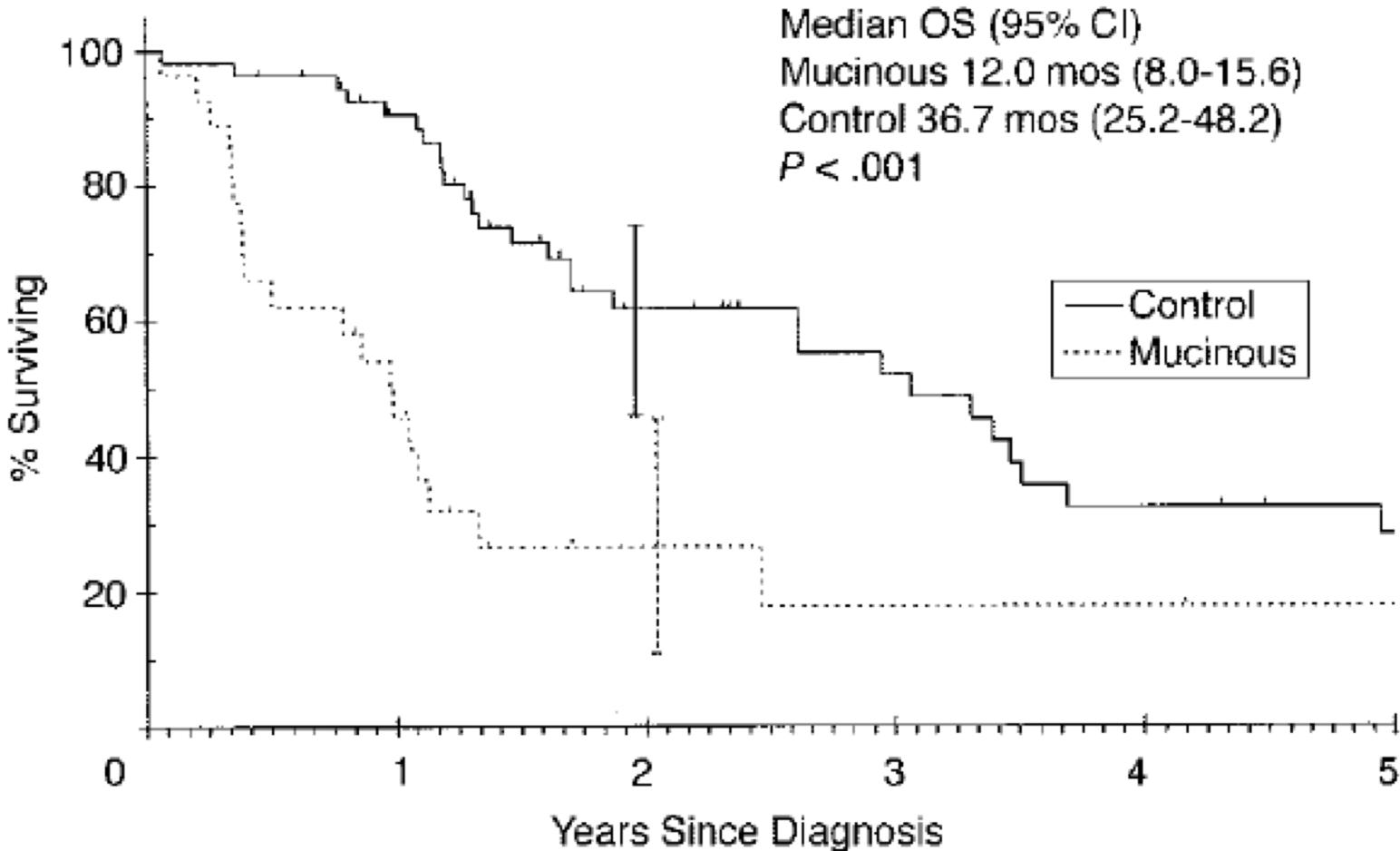
- ARID1A mutated or lost in
 - Over 40% clear cell
 - 30% endometrioid
 - Less than 1% serous
- Unknown oncogenic mechanism
 - No indication of which resulting pathways affected
 - Unclear therapeutic utility
- Diagnostic utility?
 - Not a ‘functional’ experiment

Mucinous



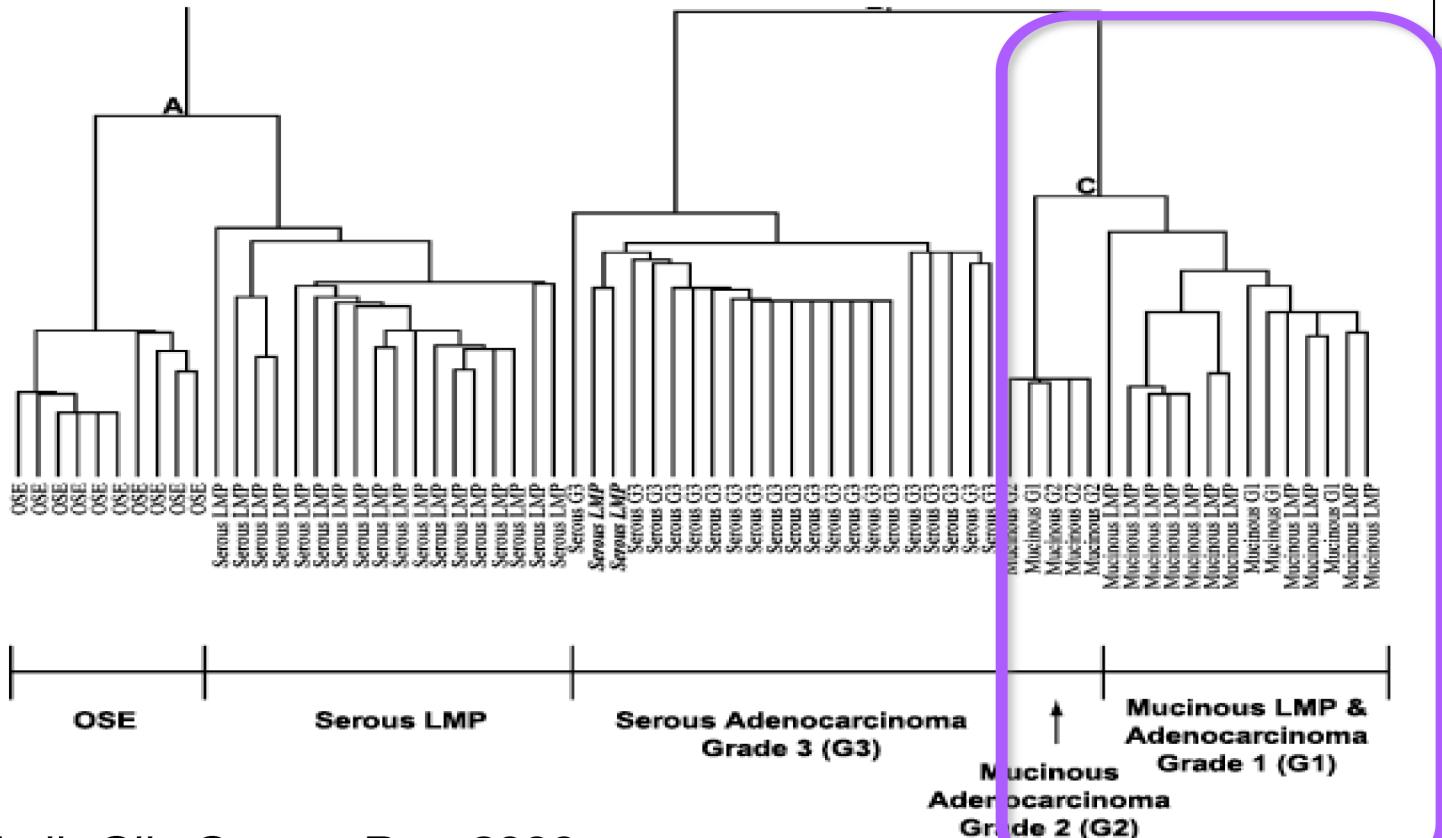


Mucinous ovarian cancer



Gene expression

Gene expression – mucinous versus serous





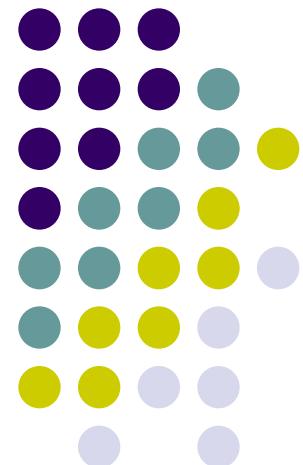
K-ras mutations

KRAS mutations - mucinous

Table 2: KRAS mutation frequencies observed in borderline malignancies

borderline			
histotype	n	mutated	% mutated
serous	20	7	35.00
endometroid	1	0	0.00
<u>mucinous</u>	6	3	50.00
unknown	2	0	0.00
total	29	10	34.48

Low grade serous





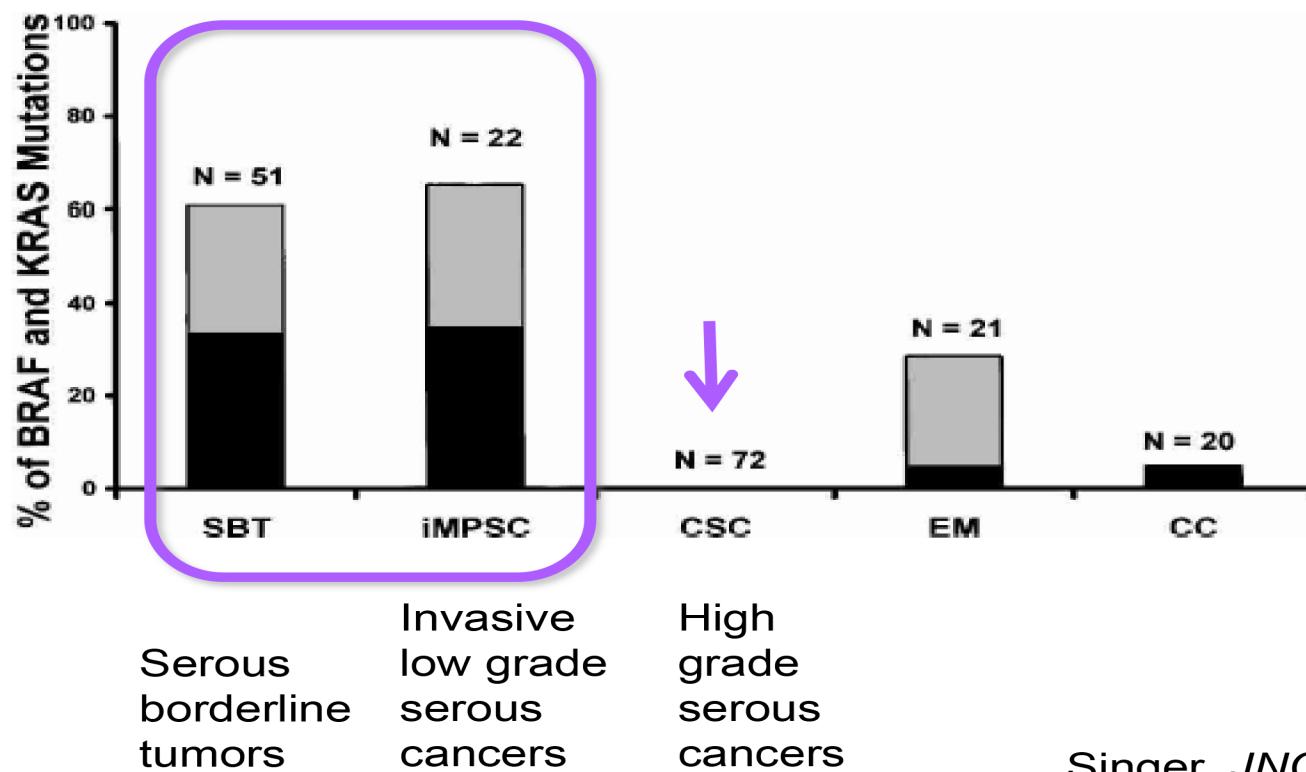
KRAS and BRAF mutations

KRAS and BRAF mutations

- BRAF codon 599
- KRAS codon 12 or 13
- 15 of 22 (68%) of low grade serous cancers
- 31 of 51 (61%) precursor lesions (SBT)
- None of 72 high grade serous cancers

KRAS and BRAF

KRAS and BRAF mutations

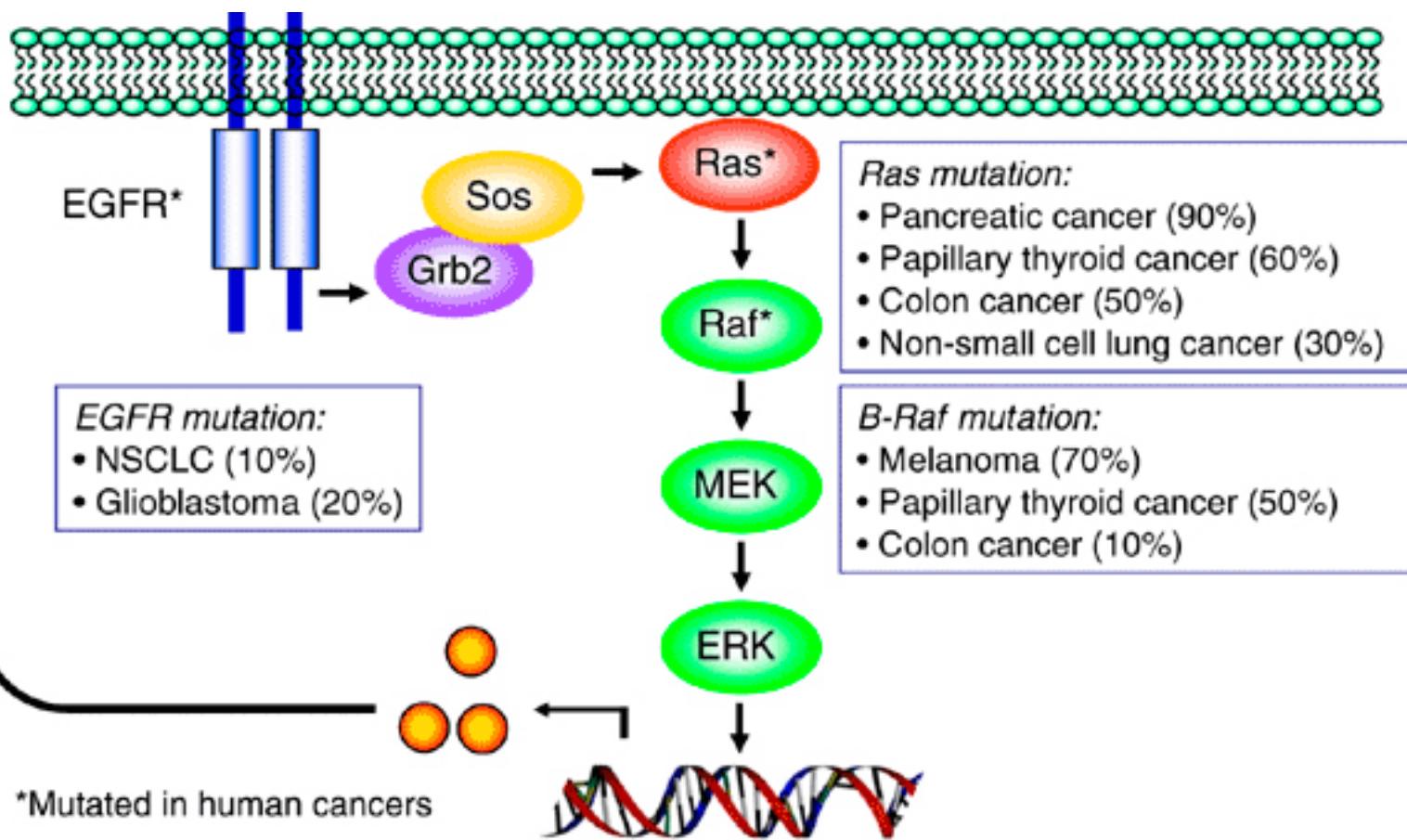


Singer, JNCI 2003



RAS signaling pathway

- a potential driver?

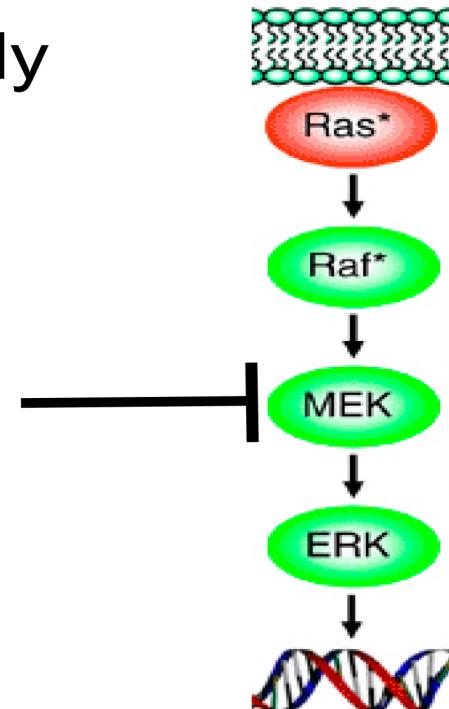




MEK inhibitor

Clinical trial: MEK inhibitor

- Recurrent Low Grade Serous ovarian cancer
- Selumetinib 50 mg twice daily
- 52 patients
 - 8 responses
 - 34 stable disease >4mo





Selumetinib responses



	Number	No tumour response	Tumour response	p value*
Total	34	27 (79%)	7 (21%)	
BRAF mutation				
No	32	25 (78%)	7 (22%)	1·000
Yes	2	2 (100%)	0	
KRAS mutation				
No	20	15 (75%)	5 (25%)	0·672
Yes	14	12 (86%)	2 (14%)	
BRAF or KRAS mutation				
No	18	13 (72%)	5 (28%)	0·405
Yes	16	14 (88%)	2 (13%)	

Data are number (%), unless otherwise indicated. *Fisher's exact test.

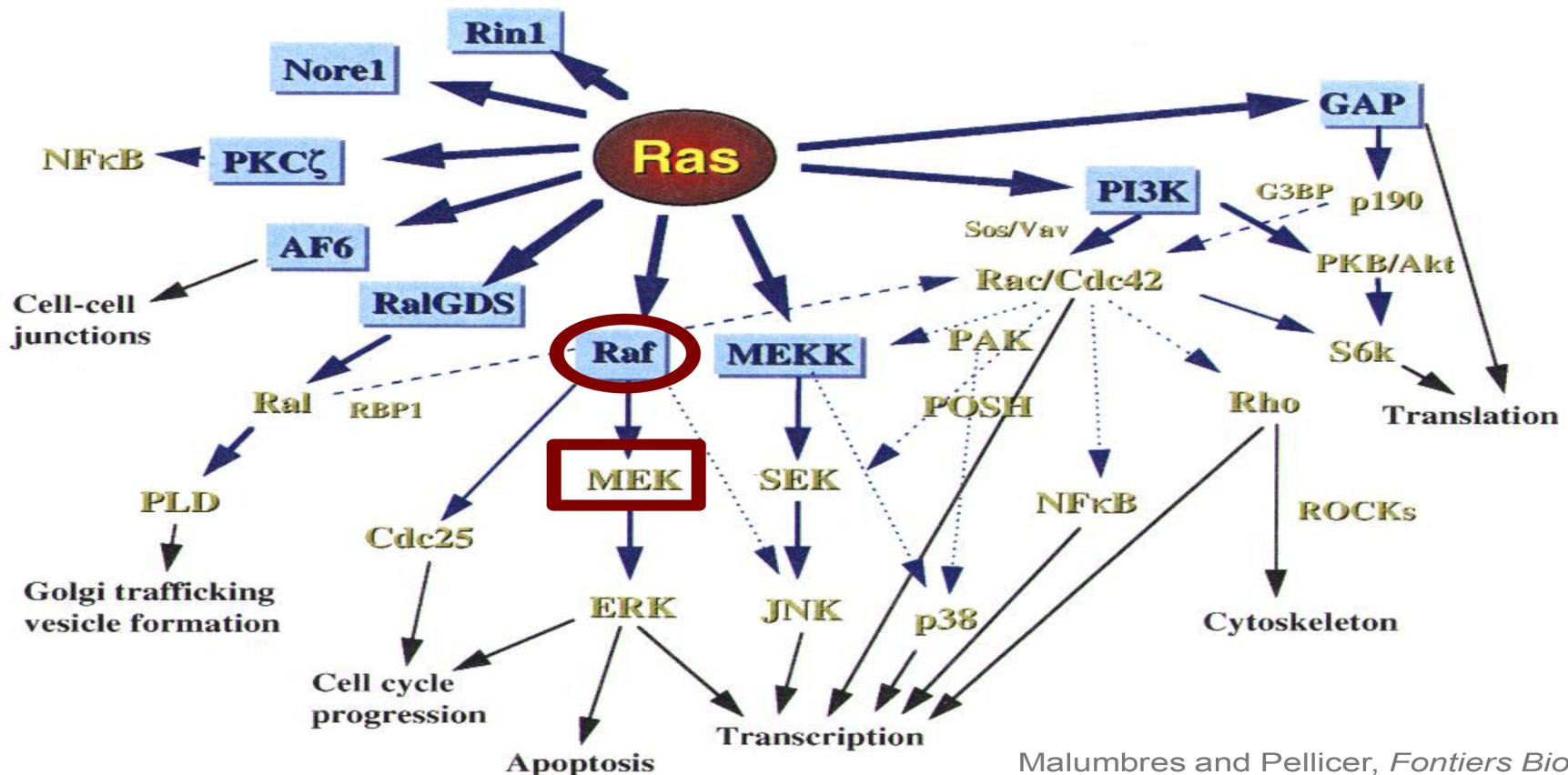
Table 8: Tumour response (complete or partial) by BRAF and KRAS mutations

Farley, *Lancet Oncol* 2013

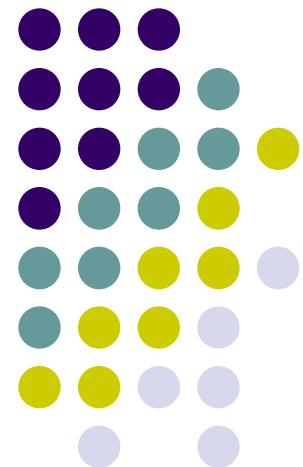
RAS signaling



RAS signaling



High grade serous





High grade serous cancers

High grade serous cancers

- **The Cancer Genome Atlas (TCGA)**
 - Clinically annotated HGS-OvCa samples
 - Identify molecular abnormalities that
 - influence pathophysiology,
 - affect outcome and
 - constitute therapeutic targets.
 - Microarray analyses: 489 HGS-OvCa tumours,
 - mRNA expression,
 - microRNA (miRNA) expression,
 - DNA copy number and
 - DNA promoter methylation for and
 - Whole exome DNA sequence: 316 samples.



High grade serous cancers

- **Sample inclusion criteria**
 - Newly diagnosed patients
 - ovarian serous adenocarcinoma
 - no prior treatment
 - companion normal tissue specimen
 - adjacent normal tissue,
 - peripheral lymphocytes,
 - or previously extracted germline DNA



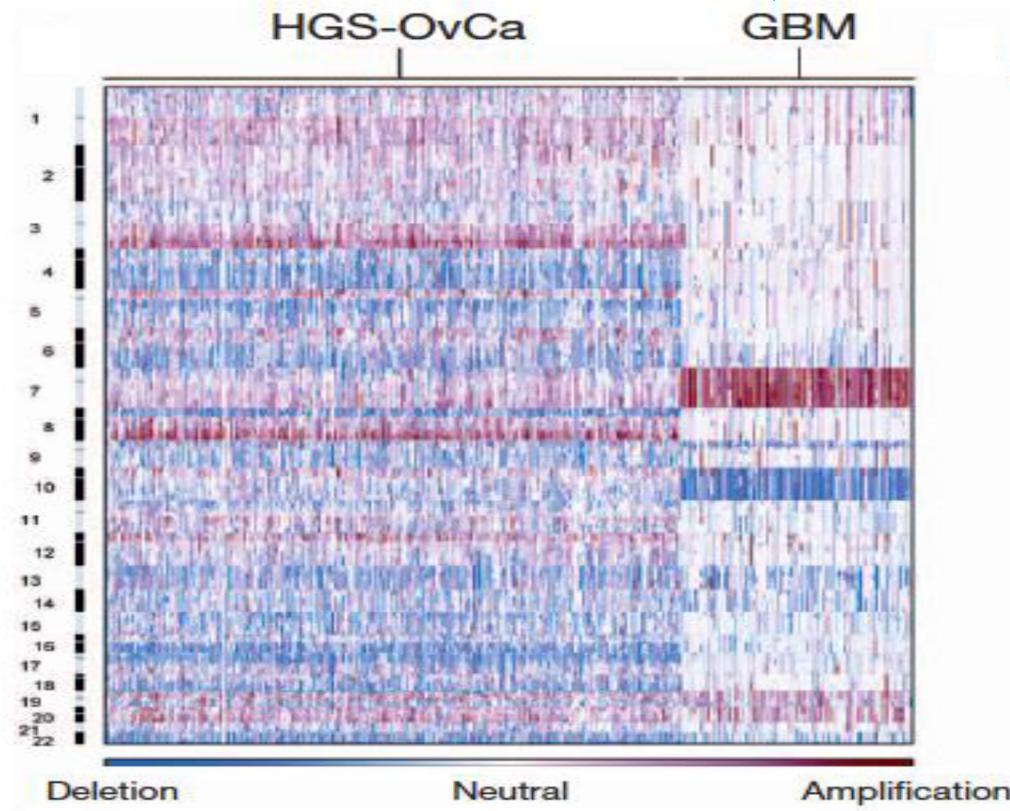
Genome copy number



Genome copy number abnormality

Copy number profiles of 489 HGS-OvCa, compared with profiles of 197 glioblastoma multiforme (GBM) tumours.

Copy number increases (red) and decreases (blue) are plotted as a function of distance along the normal genome (vertical axis, divided into chromosomes).





Mutated genes

Table 2 | Significantly mutated genes in HGS-OvCa

Gene	No. of mutations	No. validated	No. unvalidated
<i>TP53</i>	302	294	8
<i>BRCA1</i>	11	10	1
<i>CSMD3</i>	19	19	0
<i>NF1</i>	13	13	0
<i>CDK12</i>	9	9	0
<i>FAT3</i>	19	18	1
<i>GABRA6</i>	6	6	0
<i>BRCA2</i>	10	10	0
<i>RB1</i>	6	6	0

Validated mutations are those that have been confirmed with an independent assay. Most of them are validated using a second independent whole-genome-amplification sample from the same tumour. Unvalidated mutations have not been independently confirmed but have a high likelihood to be true mutations. An extra 25 mutations in *TP53* were observed by hand curation.



Altered pathways

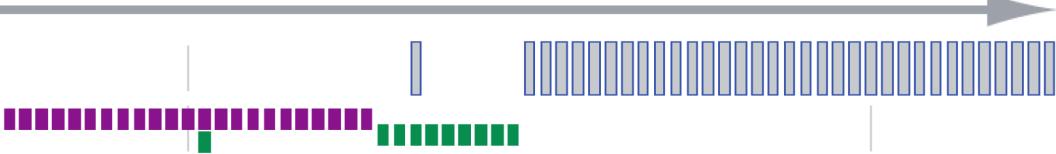
Altered pathways in HGS-OvCa

HR alterations

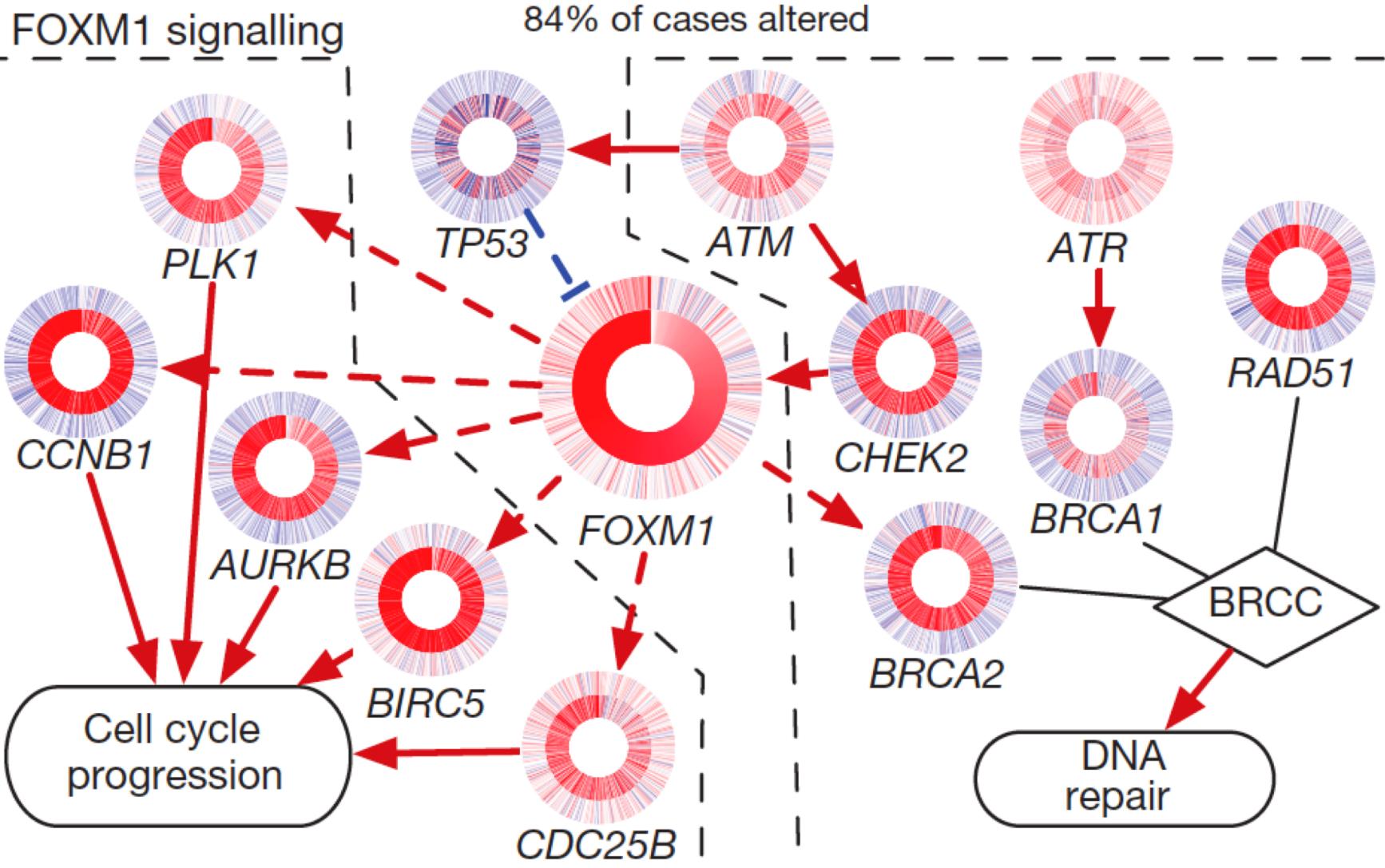
BRCA altered cases, $N = 103$ (33%)

BRCA1

BRCA2



Altered pathways in HGS-OvCa



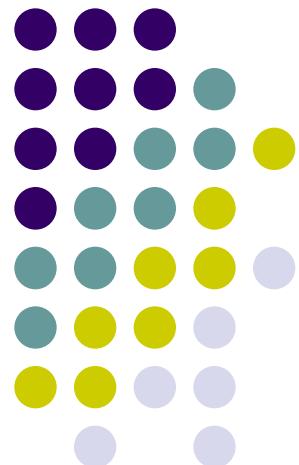


TCGA – what next?

- New **therapeutic** approaches?
 - 50% with HR defects : **PARP inhibitors**
 - commonly deregulated pathways: RB, RAS/PI3K, FOXM1, NOTCH, provide opportunities for therapeutic treatment
 - Inhibitors exist for 22 genes in regions of recurrent amplification
- aberrant genes or **networks**: targeted therapies selected to be effective ...

Targeting deficient Homologous Recombination

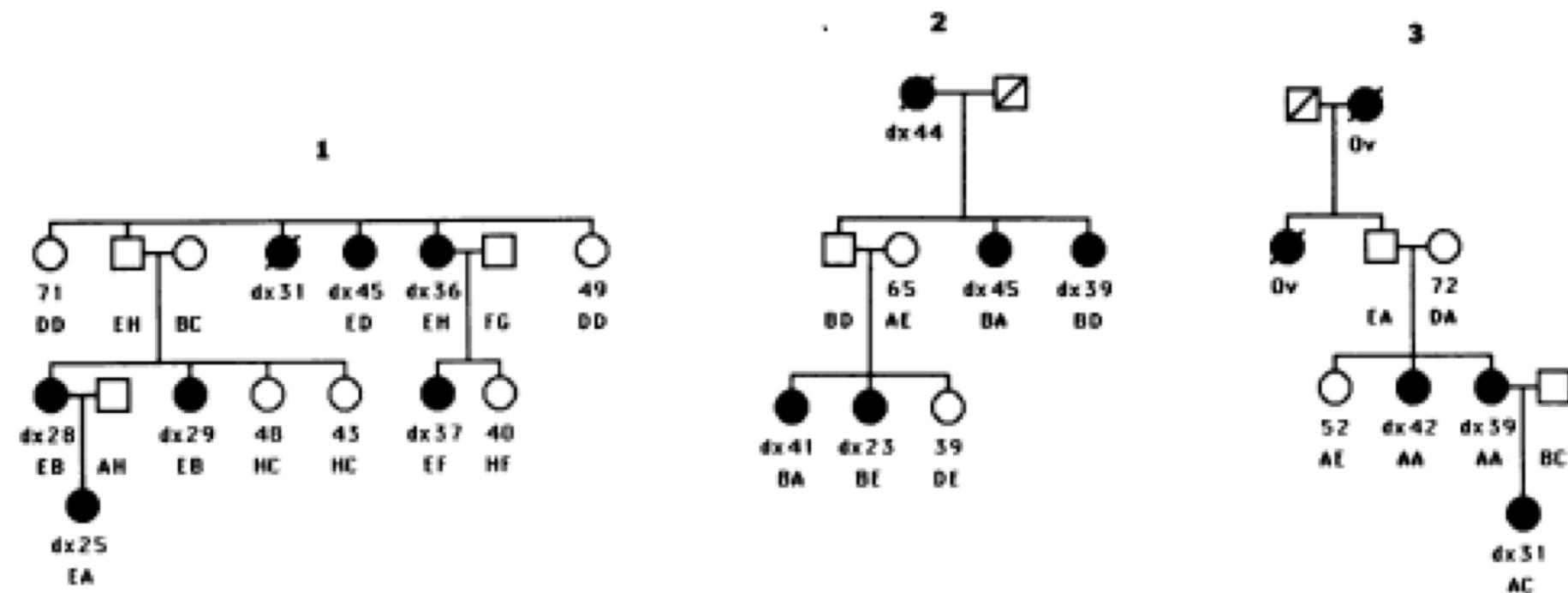
PARP inhibitors





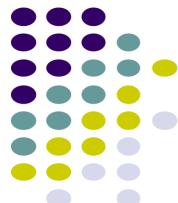
BRCA mutations

- Hall...King, *Science*, 1990

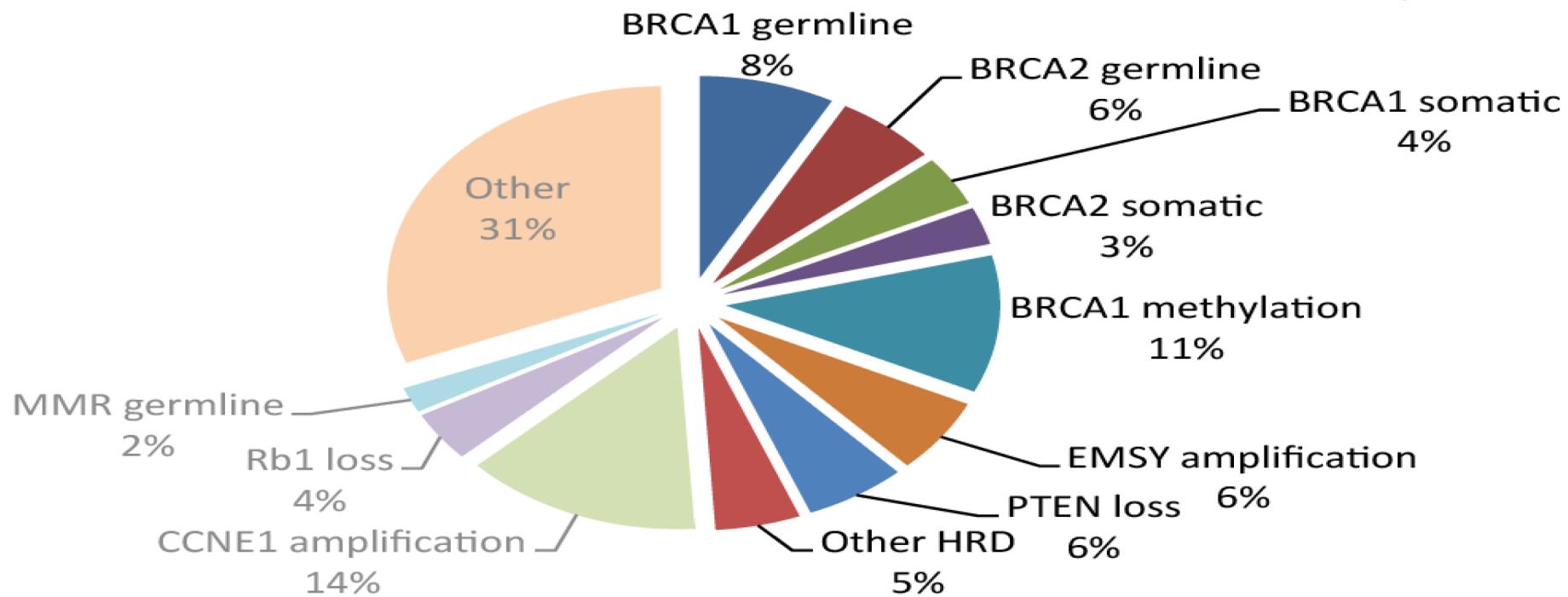




High grade serous cancers



High grade serous cancers

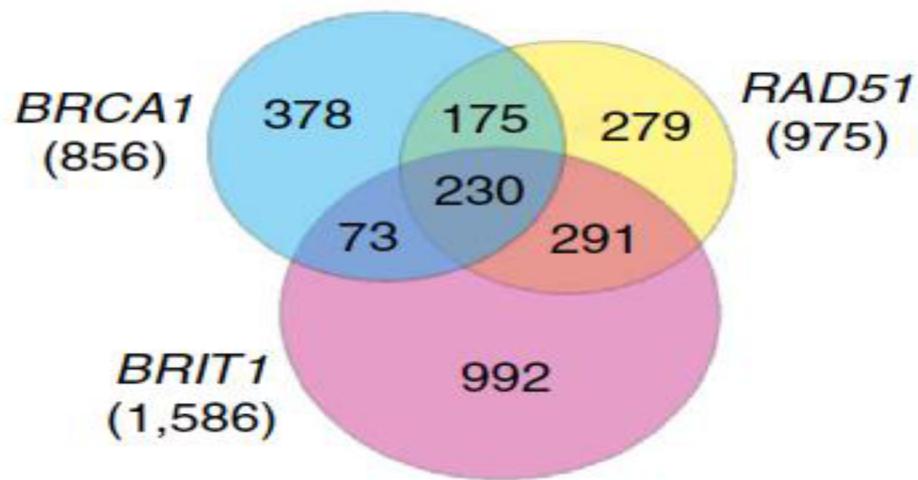


* HRD, homologous recombination defect

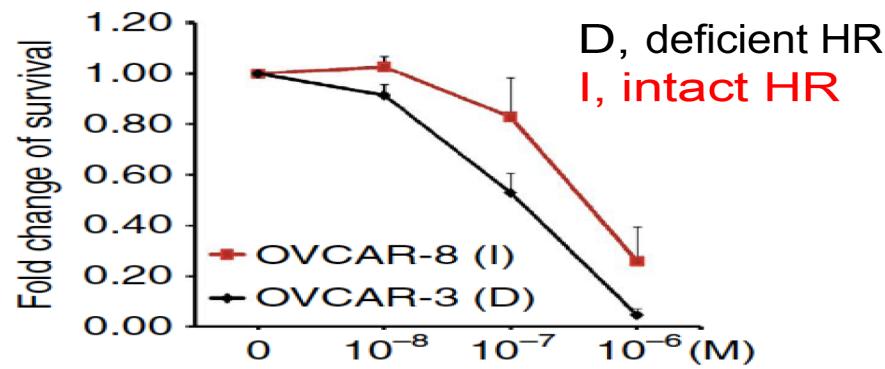
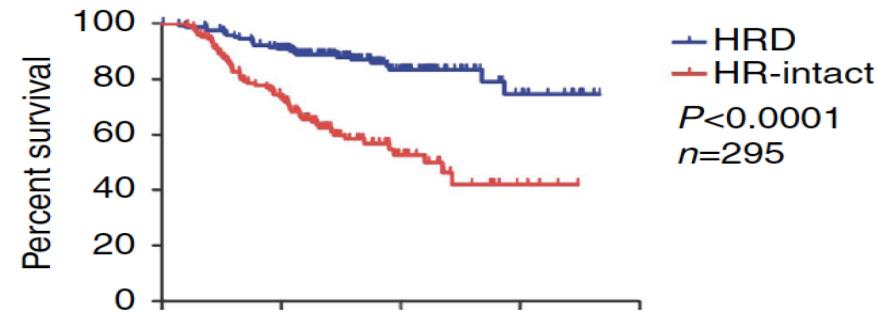
BRCA mutations

BRCA mutations... and beyond

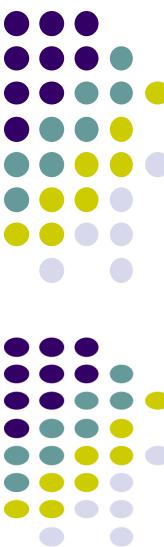
Genes associated with mutations in Homologous Recombination machinery



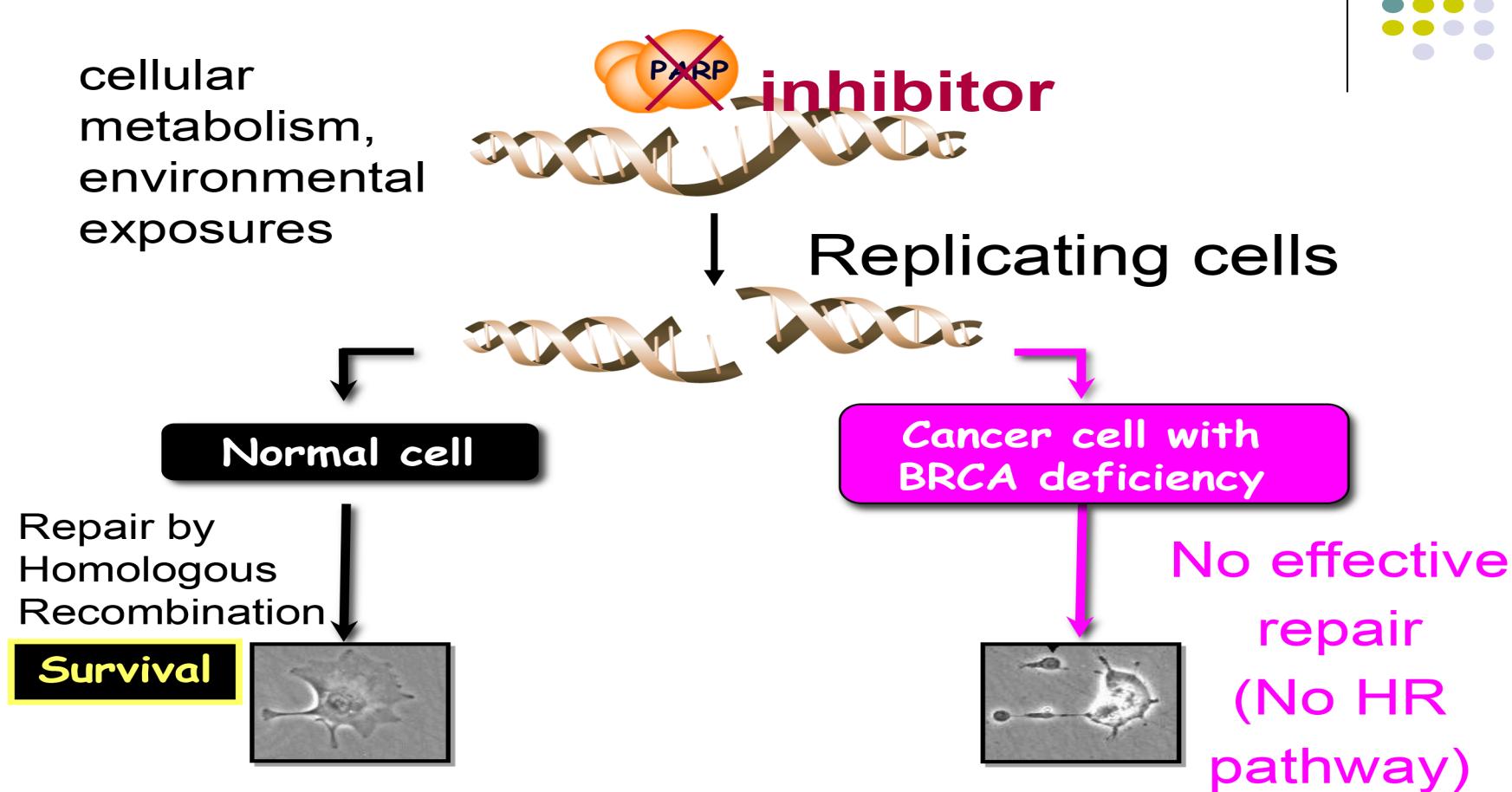
Peng et al, *Nat Comm*, 2014



PARP inhibition



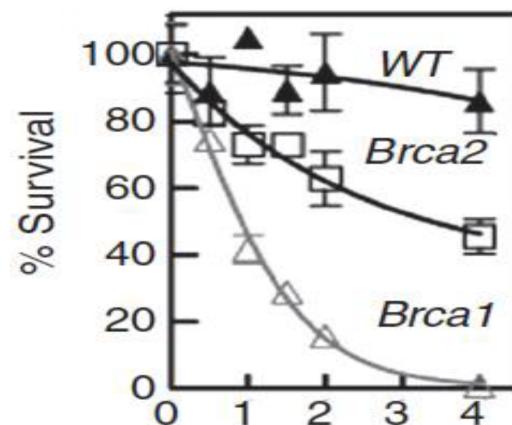
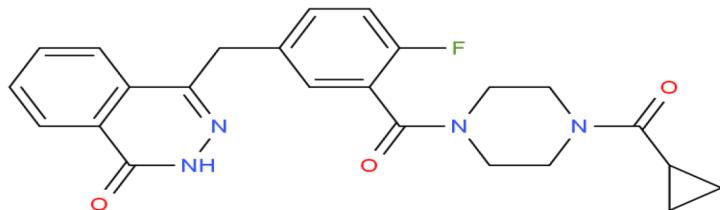
PARP inhibition: BRCA-mutant cancers



PARP inhibitor

PARP inhibitor

- Olaparib (AZD2281)
 - novel, orally active **PARP inhibitor**
 - synthetic lethality in homozygous BRCA-mut cells



Phase I/Ib study

Phase I/Ib Study of Olaparib and Carboplatin

Cohort 1

Br/Ov cancers
BRCA mutant
BRCApro ≥ 30%

(Lee, JNCI 2014)

Cohort 2

TNBC
BRCA normal
BRCApro ≤ 10%

(Chiou, AACR 2014)

Cohort 3

Serous Ovarian
BRCA normal
BRCApro ≤ 20%

(Chiou, ASCO 2015)

- Olaparib 400mg twice daily (days 1-7)
- Carboplatin AUC 5 (every 21 days)

- Olaparib 400mg twice daily (days 1-7)
- Carboplatin AUC 4 (every 21 days)

- Olaparib 400mg twice daily (days 1-7)
- Carboplatin AUC 4 (every 21 days)

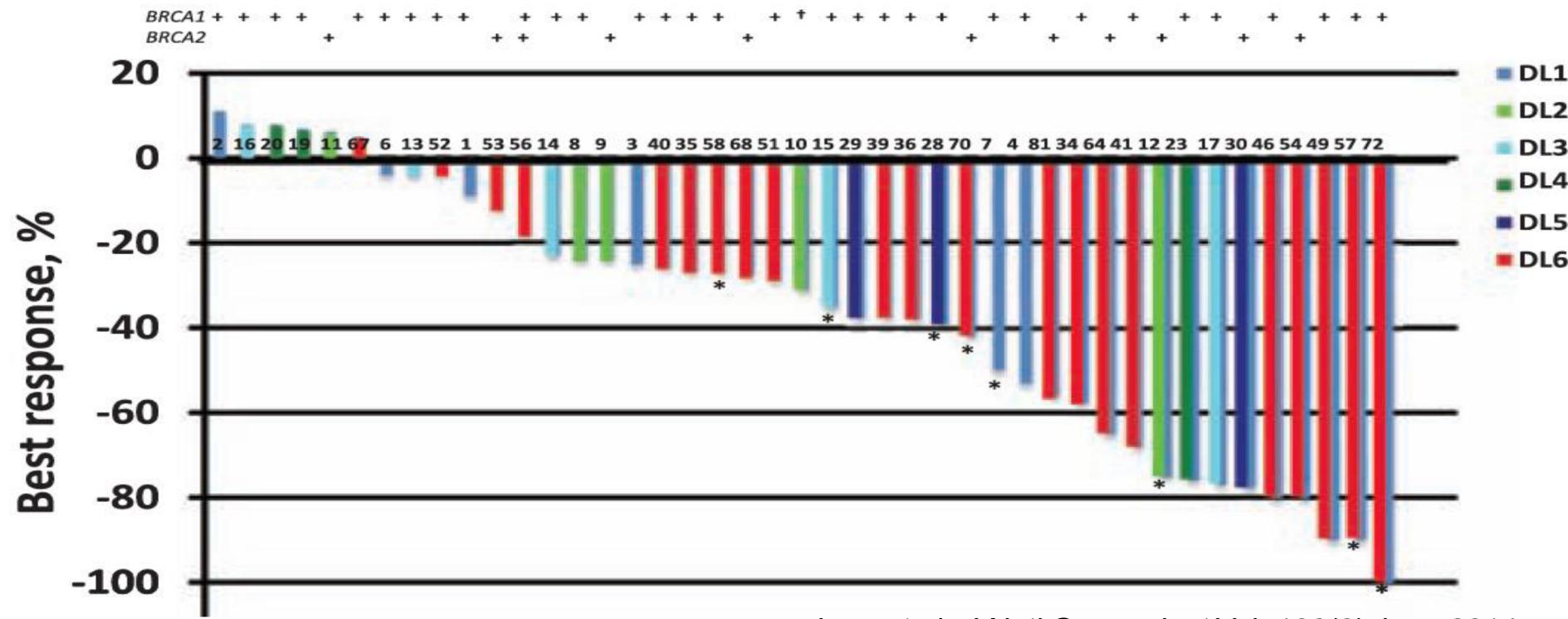
Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer



- **Results:** 45 enrolled patients
 - 37 ovarian cancer
 - 8 breast cancer
- Phase 1 dose escalation = 30 patients
- Phase 1b expansion = 15 patients
- **MTD** = Carboplatin AUC5 on day 1 + Olaparib 400mg twice daily on days 1-7, every 21 days

Phase 1b Study

**Phase Ib Study of Olaparib and Carboplatin
in BRCA1 or BRCA2 Mutation-Associated
Breast or Ovarian Cancer**





Phase 1b study

Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

Best response	Ovarian cancer (n = 34)†	
	No. (%)	Median duration in months (range)
CR	0	
PR	15 (44.1)	16 (4 to >45)
SD ≥ 4 mo	13 (38.2)	11 (6 to 24)
PD	6 (17.6)	
Overall response rate		15/34 (44.1)
Clinical benefit rate		28/34 (82.3)

Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

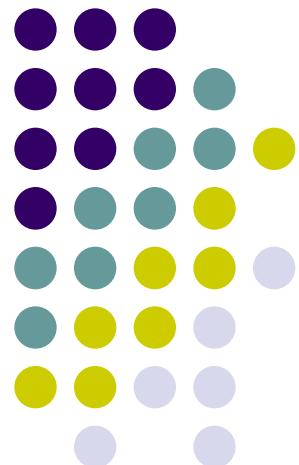


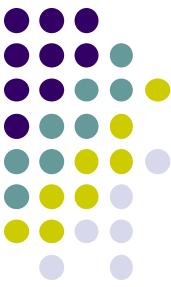
● Conclusions:

- Oral olaparib is well tolerated in combination with carboplatin
- Highly active in advanced, chemotherapy-refractory BRCA-deficient cancer
- Greater activity seen at the higher dose
- Positive proof of the concept of the activity and tolerability of **genetically defined targeted therapy** with olaparib in BRCA-deficient cancers
- Results of sporadic HGSOC cohort to be presented at ASCO meeting 2015

Exploration of new targets

Functional Genomics





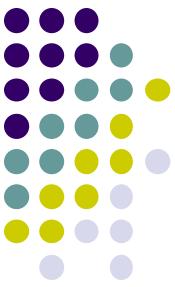
“Actionable” mutations

- Commercially available testing
 - e.g., Caris, Foundation One
 - Report “possible” or “unlikely” benefit
- “Basket” clinical trials
 - e.g., NCI-MPACT
 - Assign treatment based on mutation
- Typically no functional link



“Actionable” mutations

- “...depends in large part on the strength of the data linking the target and targeted therapy.”
- “For this trial design to work, two key conditions must be met:
 - the tumor must depend on the target pathway, and
 - the targeted therapy must reliably inhibit the target.”
- “Achieving both goals can be a matter of some complexity.”



“Actionable” targets

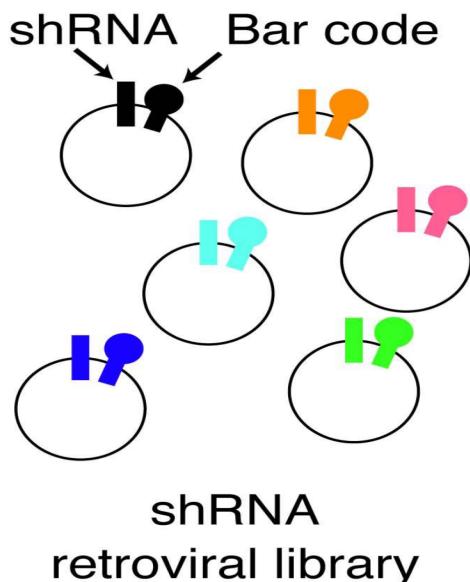
- Need a functional experiment
- Functional genomics



Functional genomics

Using a functional genomics screen to identify targets

Creation of an Inducible shRNA Retroviral Library for Functional Genomics Studies of Cancer Phenotypes

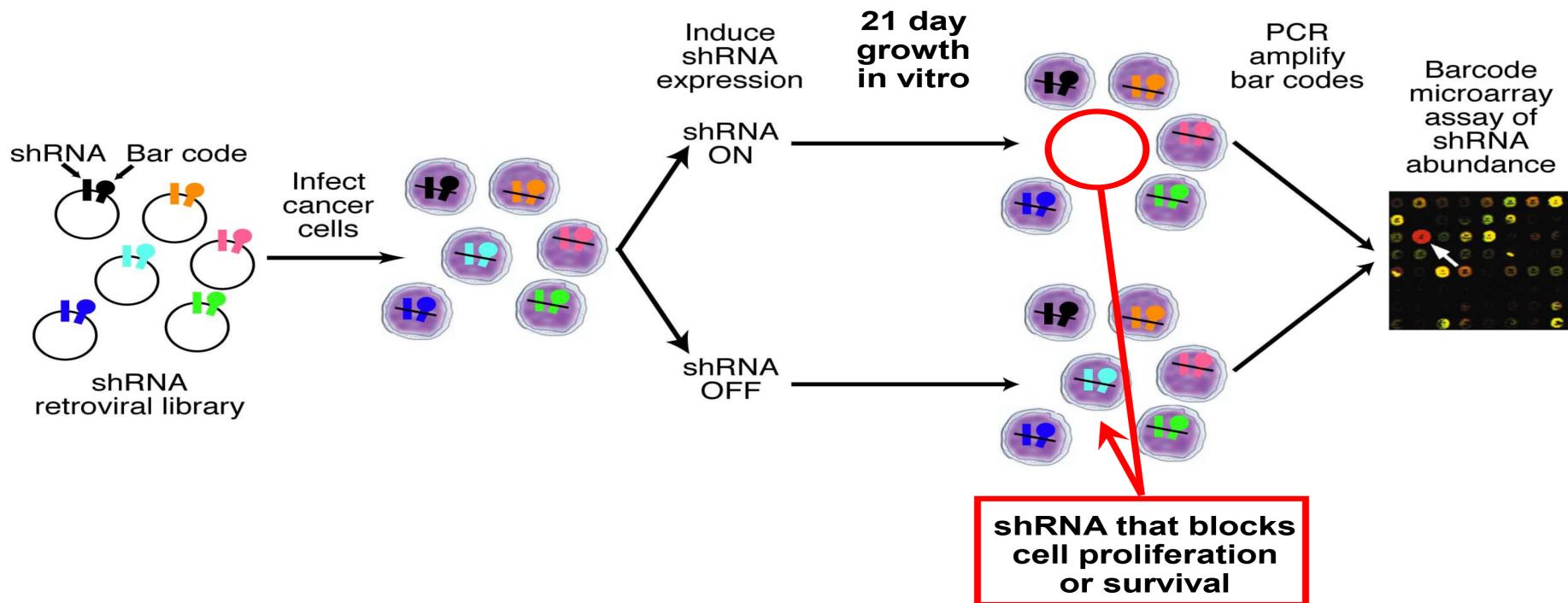


- shRNAs targeting **2500** human genes
- 3 shRNA constructs per gene
- All sequence verified
- All containing identified **60-mer bar code sequence**
- shRNA expression is inducible by doxycycline
- Library target genes:
 - All protein kinases
 - All PI3 kinase
 - All deubiquitinating enzymes
 - NF- κ B pathway regulators
 - Differentially expressed genes among lymphoma types
 - Apoptosis regulators, oncogenes, tumor suppressors

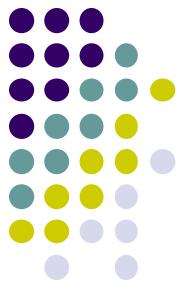


shRNA Library Screen

shRNA Library Screen for Genes Controlling Cancer Cell Proliferation and Survival

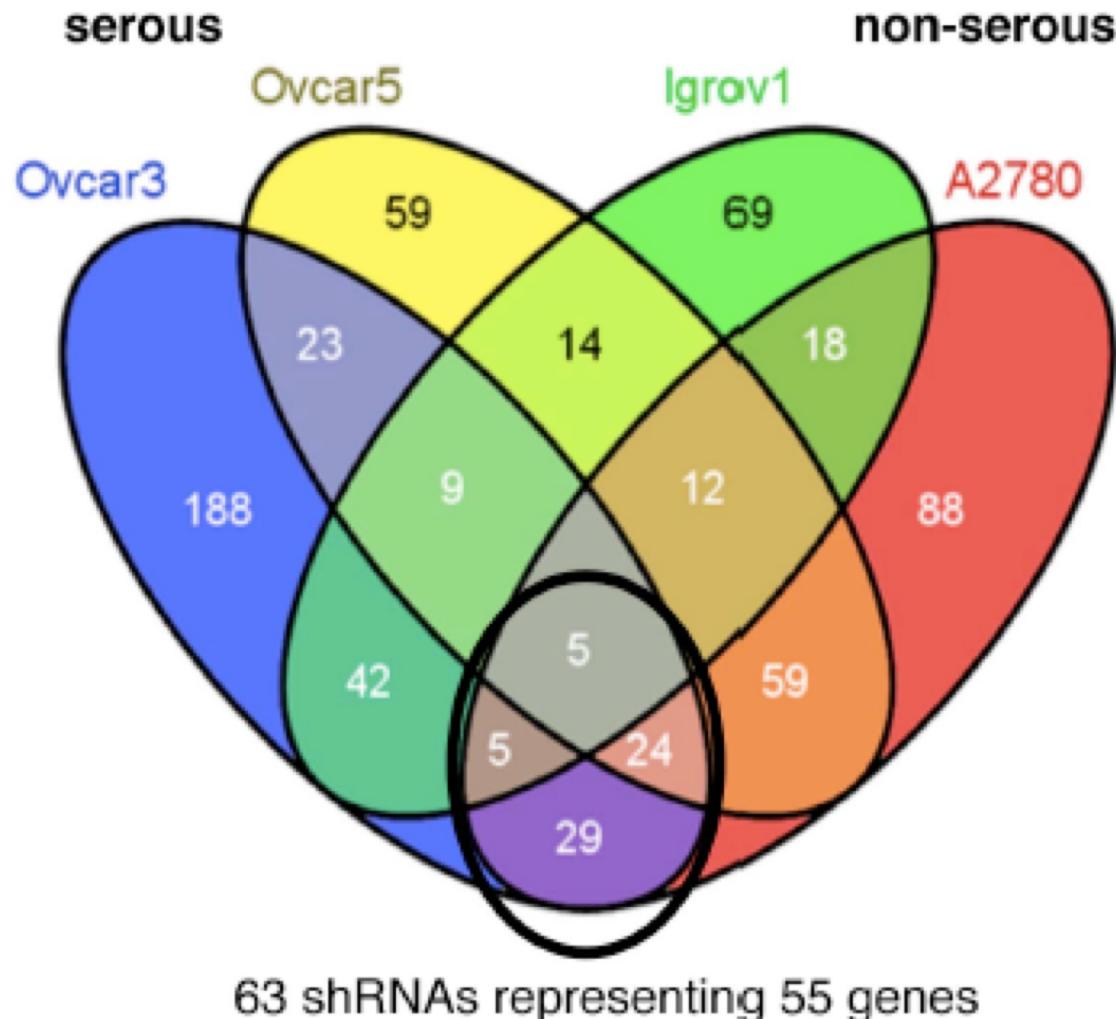


Functional Genomics of ovarian cancer



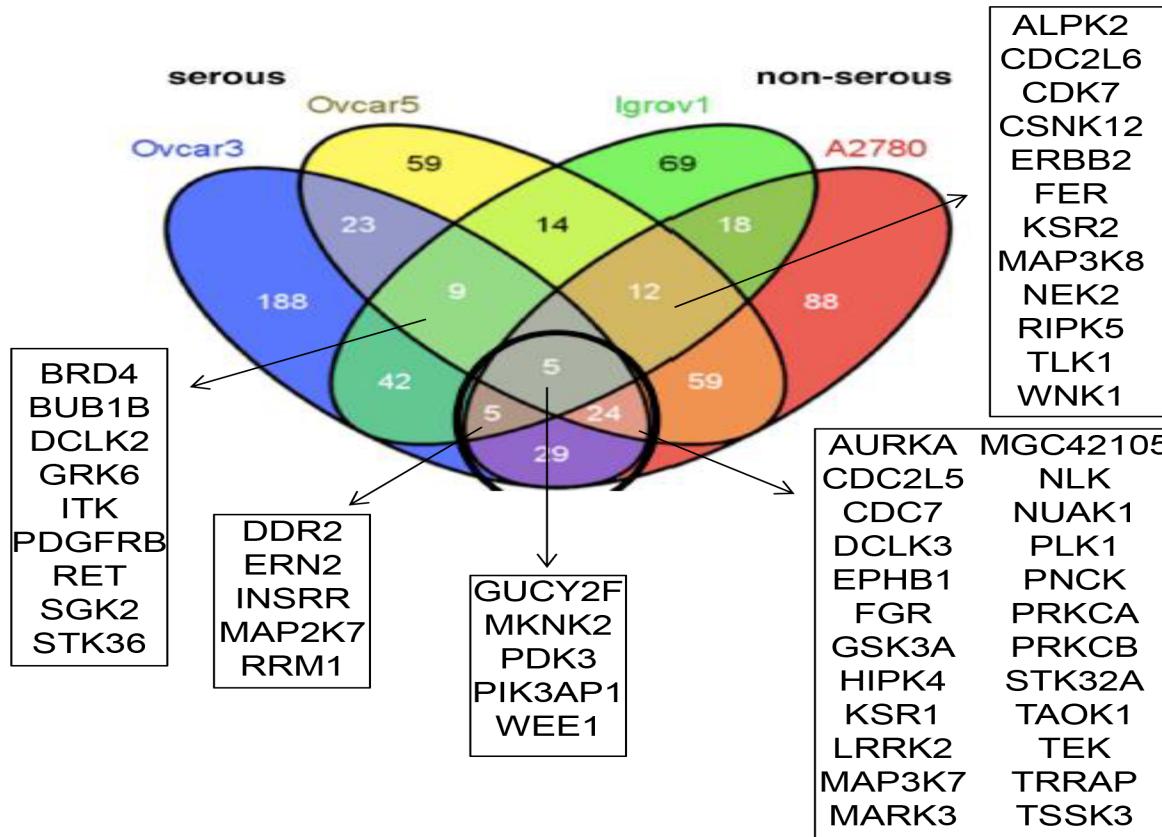
- Four ovarian cancer cell lines
 - OVCAR3 – serous
 - OVCAR5 – serous
 - Igrov1 – non-serous
 - A2780 – non-serous

Common targets in ovarian cancer – “drivers”?

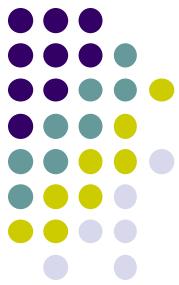


Common targets

Common targets in ovarian cancer – “drivers”?



Functional genomics of ovarian cancer

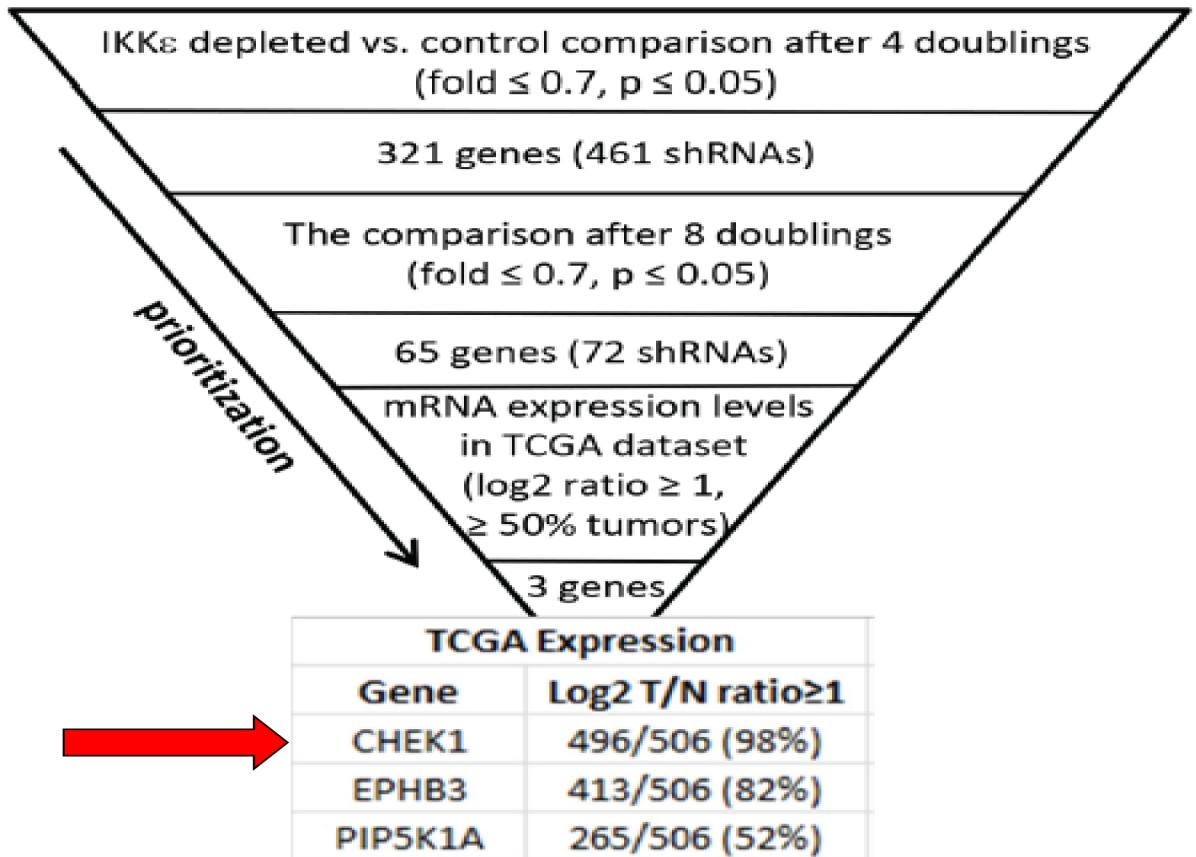


- Following up in
 - 6 additional cell lines
 - 2 different RNAi constructs
 - Select “druggable” targets
- Focused functional screens
 - Specific subgroup of serous ovarian cancer
 - NF-kappaB signaling pathway

CHEK1

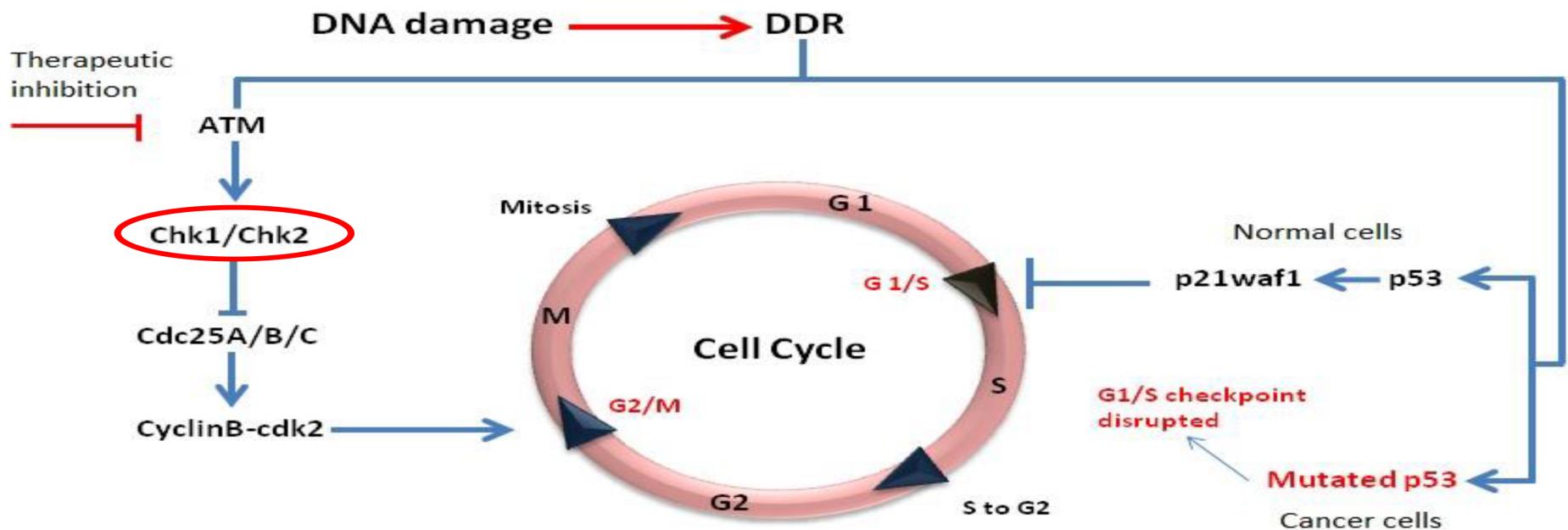
CHEK1

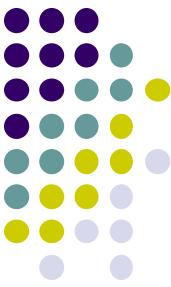
- Highly synergistic with IKK ϵ
- Over-expressed in nearly all ovarian cancers



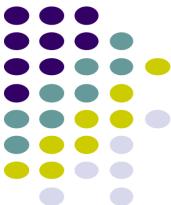
CHEK signaling

CHEK signaling



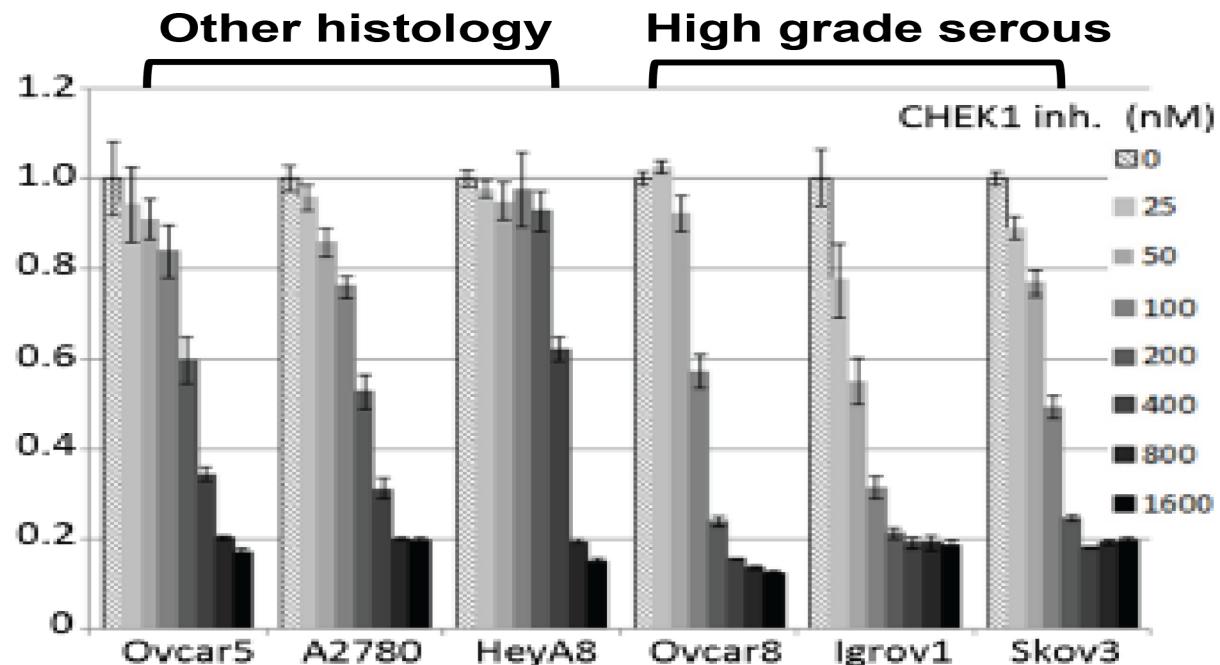


CHEK inhibitor



CHEK inhibitor

- Most potent in HGSOC





CHEK inhibitor

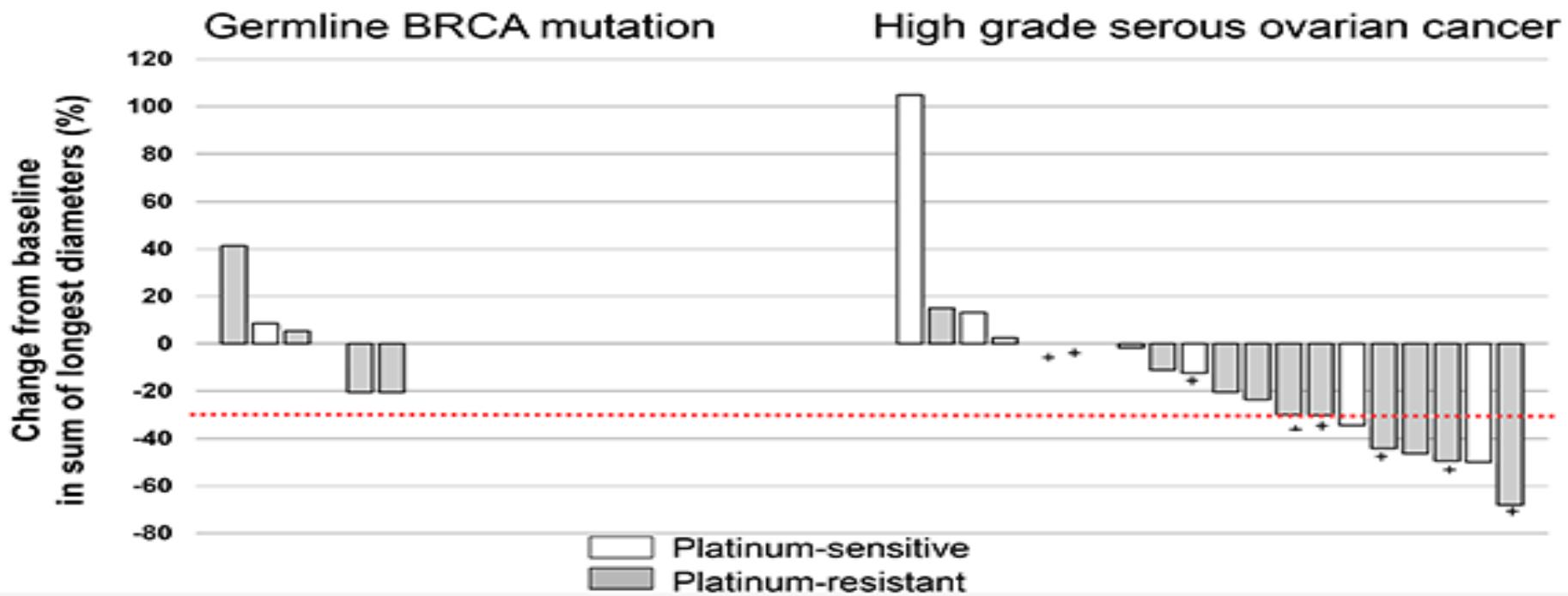
- Clinical trial ongoing
 - NCT02203513
 - Promising results in High grade serous non BRCA
- Highlighted by a Functional Genomics approach



Best response on clinical trial



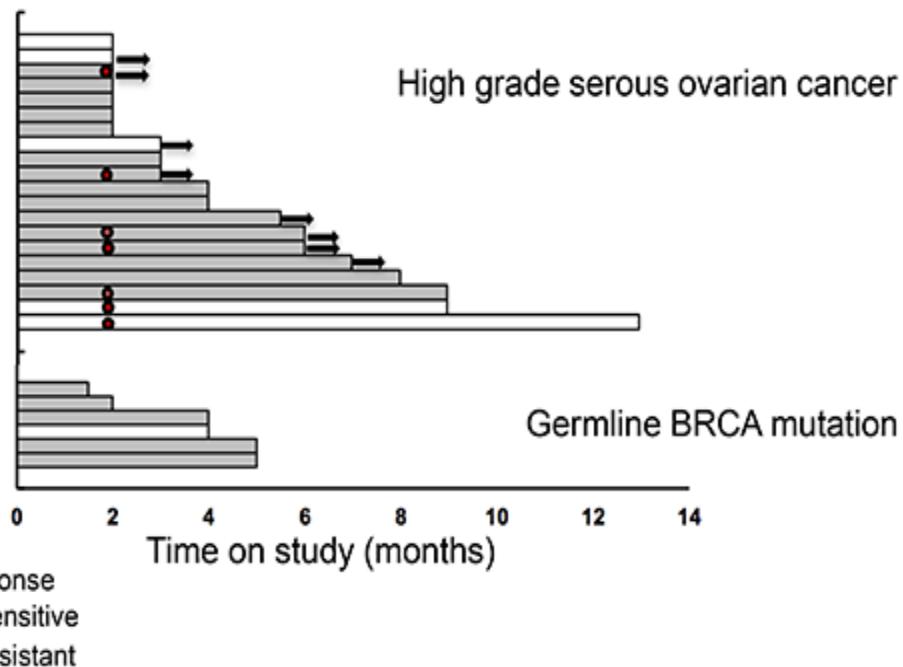
Best response on clinical trial





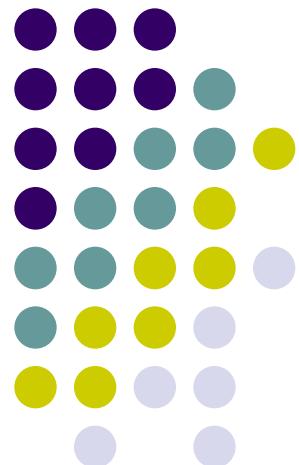
Duration on clinical trial

Duration on clinical trial



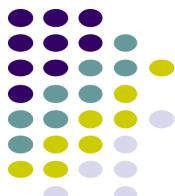
Ovarian cancer genomics

Summary

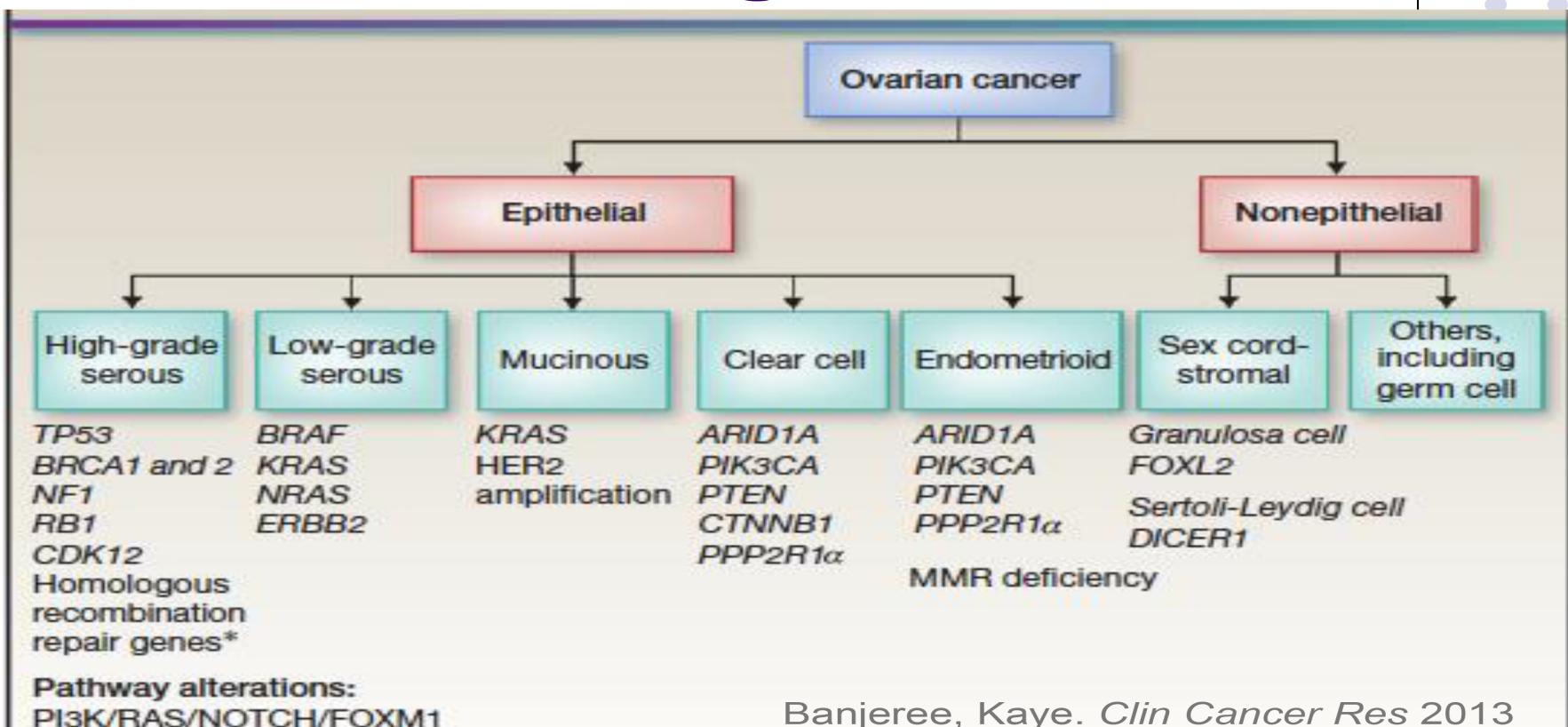




Ovarian cancer genomics



Ovarian cancer genomics





Functional Genomics

- 1981 – Shih – discovery of Her2/neu

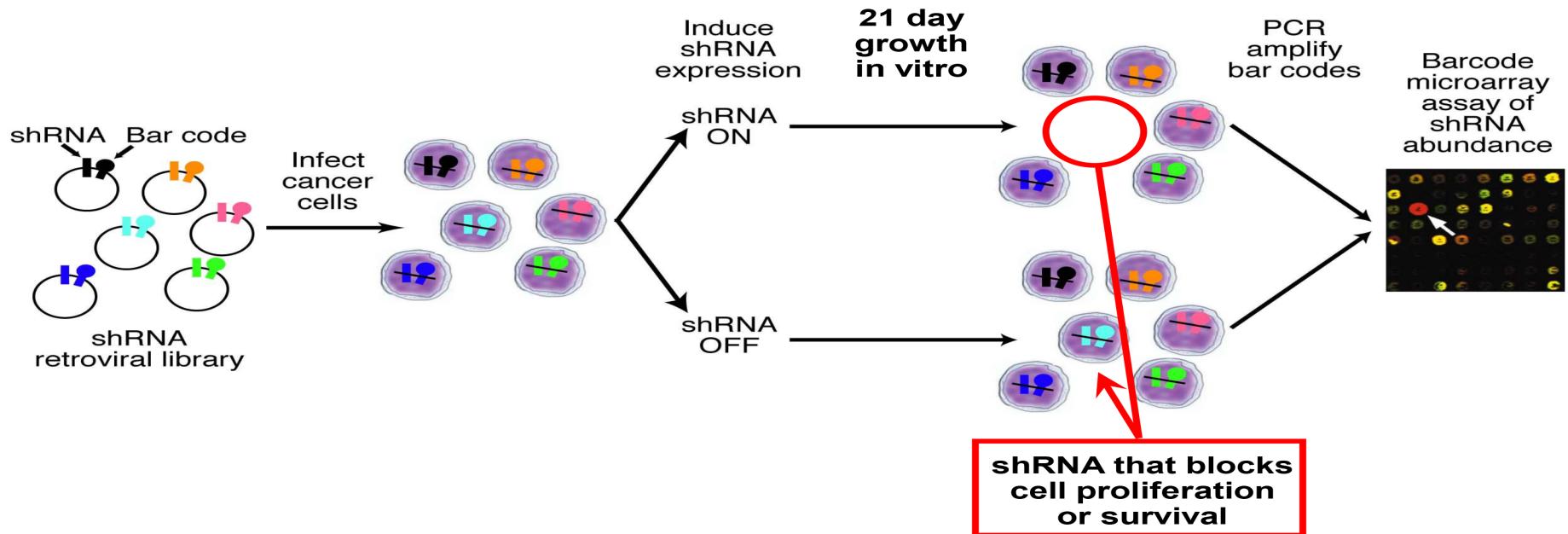
**Transforming genes of carcinomas
and neuroblastomas
introduced into mouse fibroblasts**

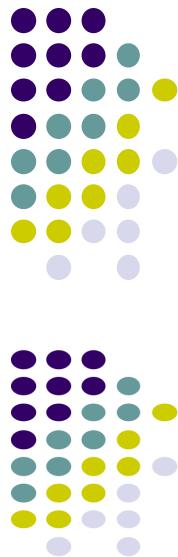
**Chiaho Shih, L. C. Padhy, Mark Murray
& Robert A. Weinberg**



Controlling genes

shRNA Library Screen for Genes Controlling Cancer Cell Proliferation and Survival





Ovarian cancer and genomics

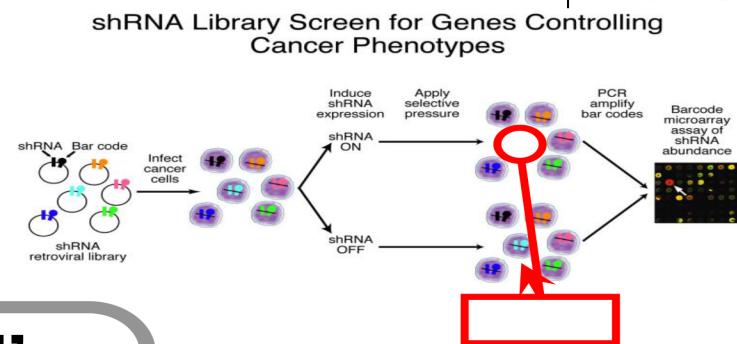
Ovarian Cancer in the Genomics Era

Functional genomic screen

“Driver”
aberration/pathway

Transforming genes of carcinomas
and neuroblastomas
introduced into mouse fibroblasts

Chiaho Shih, L. C. Padhy, Mark Murray
& Robert A. Weinberg



Clinical trial

Women's cancer team

Women's Cancer Team:

Stan Lipkowitz, MD, PhD

Jung-Min Lee, MD

Alexandra Zimmer, MD

Victoria Chiou, MD

Ciara O'Sullivan, MD

Anne Noonan, MD

Elise C. Kohn, MD

Nicole Houston, RN

Irene Ekwede, RN

MOS Fellows and Nursing Staff

Translational scientists:

Lidia Hernandez, MS

Marianne Kim, PhD

Carrie House, PhD

Kristen Bunch, MD

Collaborators:

Lou Staudt, MD, PhD

George Wright, PhD

Funding:

**National Cancer
Institute, IRP**

**Women's Cancer
Foundation**

Patients and their families